CONTENTS

The American Journal of Medicine

VOL. VIII MAY, 1950 No. 5

SYMPOSIUM ON VIRAL HEPATITIS

Foreword . . . . . W. PAUL HAVENS, JR. 559

A Note on the History of Epidemic Viral Hepatitis in Germany
HORACE T. GARDNER 561

Endemiologic Aspects of Hepatitis in U. S. Troops in Germany 1946–1950
JOHN R. PAUL AND HORACE T. GARDNER 565

An Evaluation of Two Screening Tests for the Detection of Early and Sub-icteric Viral Hepatitis
WILLIAM E. SWIFT, JR., WADE N. MILLER, FRANKLIN H. STREITFELD AND MARJORIE KNOWLTON 581

Serum Cholesterol and Cholesterol Esters in Viral Hepatitis
HORACE T. GARDNER, WILLIAM E. SWIFT, JR., FIRST LT. MARTIN MODICA, LEON LEVINTOW AND MARJORIE KNOWLTON 584

Factors Influencing Retention of Bromsulfalein in the Blood of Patients with Viral Hepatitis
W. PAUL HAVENS, JR., WADE N. MILLER, WILLIAM E. SWIFT, JR., HORACE T. GARDNER AND MARJORIE KNOWLTON 591

Comparison of the Thymol, Cephalin-cholesterol Flocculation and Colloidal Red Tests in Acute Viral Hepatitis
JOHN R. NEEFE, JOSEPH M. GAMBESCIA, HORACE T. GARDNER AND MARJORIE KNOWLTON 600

Oral Cholecystography in Patients with Viral Hepatitis
HARRY M. READINGER, WILLIAM E. SWIFT, JR., HORACE T. GARDNER AND JOHN A. SHEEDY 611

Contents continued on page 5

routine for discovery

The national total of undiagnosed or "unknown" diabetics may run from a million to two or even three.[1,2] Modern treatment, when promptly initiated, can do much to prevent metabolic decompensation and to minimize diabetic complications. Therefore, the clinical revealment of diabetes mellitus at an early stage is essential.

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(1) Joslin, E. P.: Postgraduate Med. 4:302 (Oct.) 1948. (2) Kemper, C. F.: Rocky Mountain M. J. 45:1092 (Dec.) 1948. (3) Pollack, H.: New York Med. 4:15 (Dec. 5) 1948.

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CONTENTS

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VOL. VIII MAY, 1950 No. 5

Contents continued from page 3

Clinical Course of Viral Hepatitis and the Effect of Exercise during Convalescence
WILLIAM E. SWIFT, JR., HORACE T. GARDNER, DOUGLAS J. MOORE, FRANKLIN H. STREITFELD AND W. PAUL HAVENS, JR. 614

The Hepatitis Center established at the 120th Station Hospital, Bayreuth, Germany, afforded a unique opportunity for the study of viral hepatitis, some 4,000 patients being treated there in the period 1947–1949. Dr. Havens has selected and edited for publication in this Symposium eight papers embodying the results of study of various aspects of viral hepatitis as there encountered. The Symposium is a notable contribution to our knowledge, epidemiologic, clinical and biochemical, of this important disease.

Clinical Studies

Myocardial Infarction without Anticoagulant Therapy. Deaths, Emboli and an Analysis of Factors Influencing Mortality
NED DOSCHER AND CHARLES A. POINDEXTER 623

A challenging paper which makes clear the number of variables affecting the survival rate after myocardial infarction and which should be taken into account in evaluating the efficacy of anticoagulant therapy in coronary thrombosis. The authors consider that the alternate case method of study does not equalize these variables.

Effect of Orally Administered Dihydroergocornine (D.H.O. 180) on Hypertension
C. T. BELLO, W. G. MOSS AND E. WEISS 634

Dihydroergocornine given orally was found not to lower the blood pressure of hypertensives; some response was noted after intravenous administration.

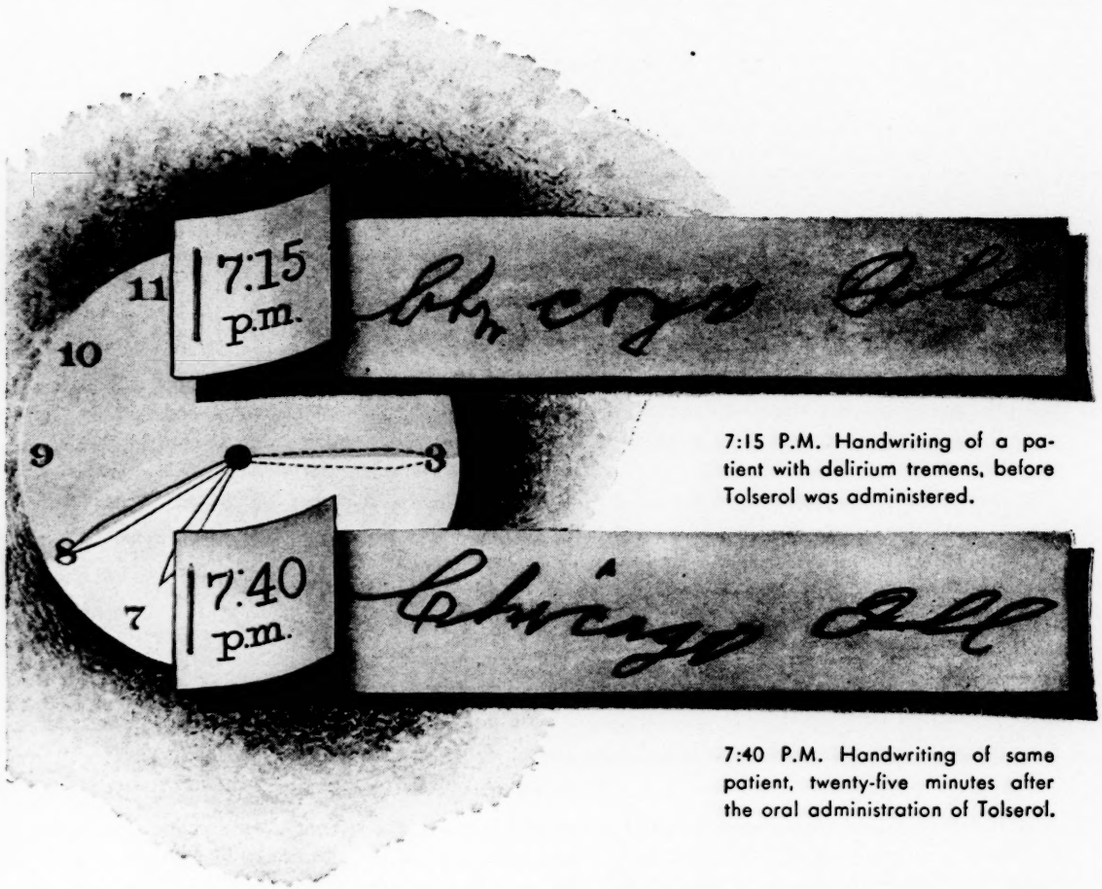
Review

Digitalis Poisoning . . . . WALTER BRODIE BURWELL AND JAMES P. HENDRIX 640

An explicit and detailed account of the clinical and electrocardiographic manifestations of digitalis poisoning, with illustrative cases to underline common errors in administration.

Contents continued on page 7

in alcoholism...



7:15 P.M. Handwriting of a patient with delirium tremens, before Tolserol was administered.

7:40 P.M. Handwriting of same patient, twenty-five minutes after the oral administration of Tolserol.

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CONTENTS

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VOL. VIII MAY, 1950 NO. 5

Contents continued from page 5

Seminars on Cancer Research

Aspects of Approaches in Experimental Cancer Chemotherapy. C. CHESTER STOCK 658

Dr. Stock here outlines the philosophy and methods of a large-scale, long-term experimental cancer chemotherapy program. The complexities of the problem, the painstaking and varied technics required, the results thus far obtained are all indicated. The important point brought out is that suitably designed animal tests can serve to screen compounds for use in human malignancy.

Case Reports

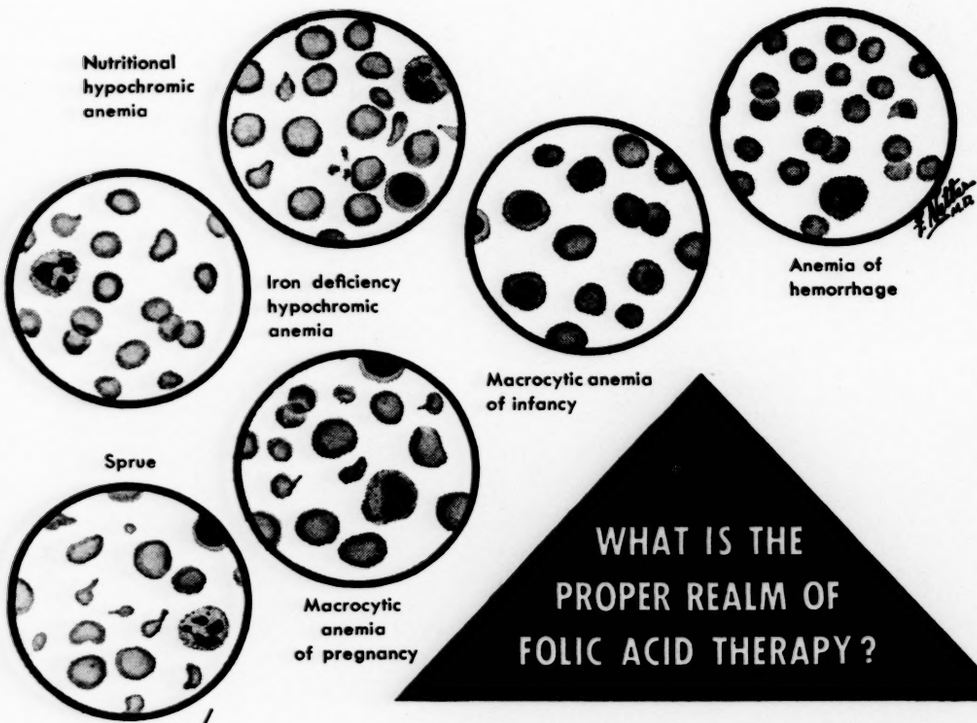
Cirrhosis of the Liver Following Infectious Mononucleosis
SIDNEY LEIBOWITZ AND HENRY BRODY 675

This intriguing case ot cirrhosis following and probably related, in part, to infectious mononucleosis is presented as part of the whole problem of liver involvement in that disorder.

Nephrotic Syndrome with Exaggerated Premenstrual Water and Salt Retention
RICHARD W. LIPPMAN 686

Dr. Lippman's interesting observation in this case of nephrosis may be duplicated in occasional other cases.

Book Reviews 688



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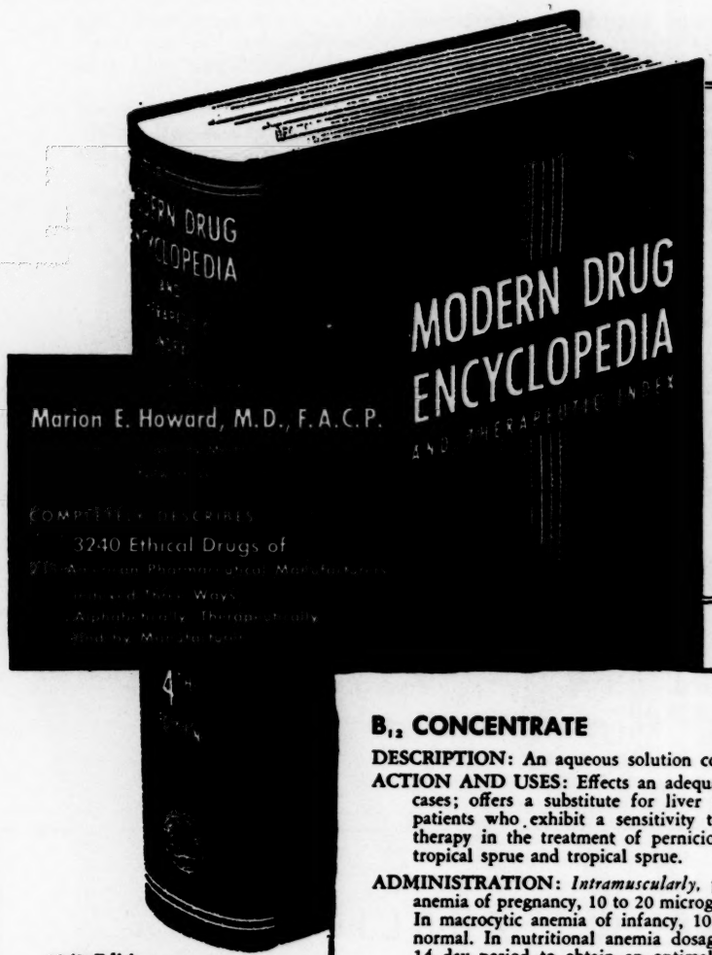

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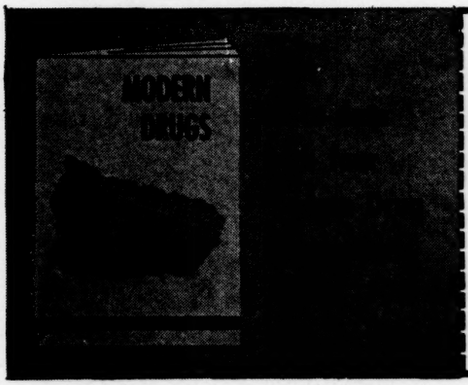

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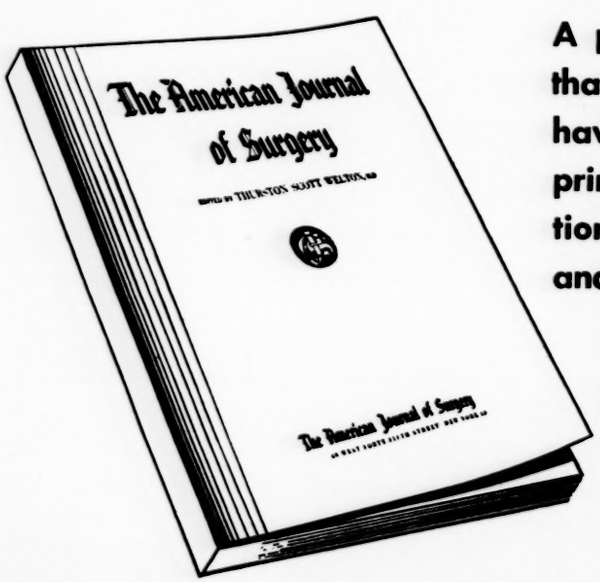

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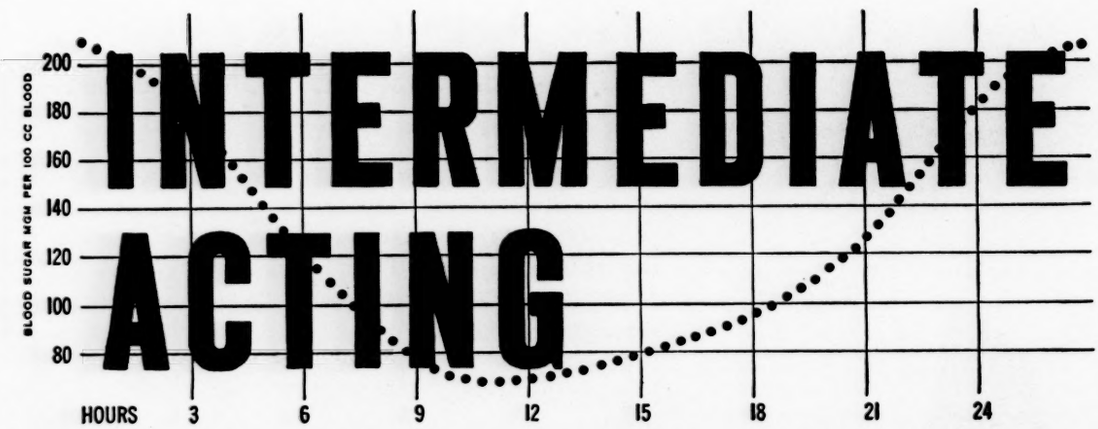

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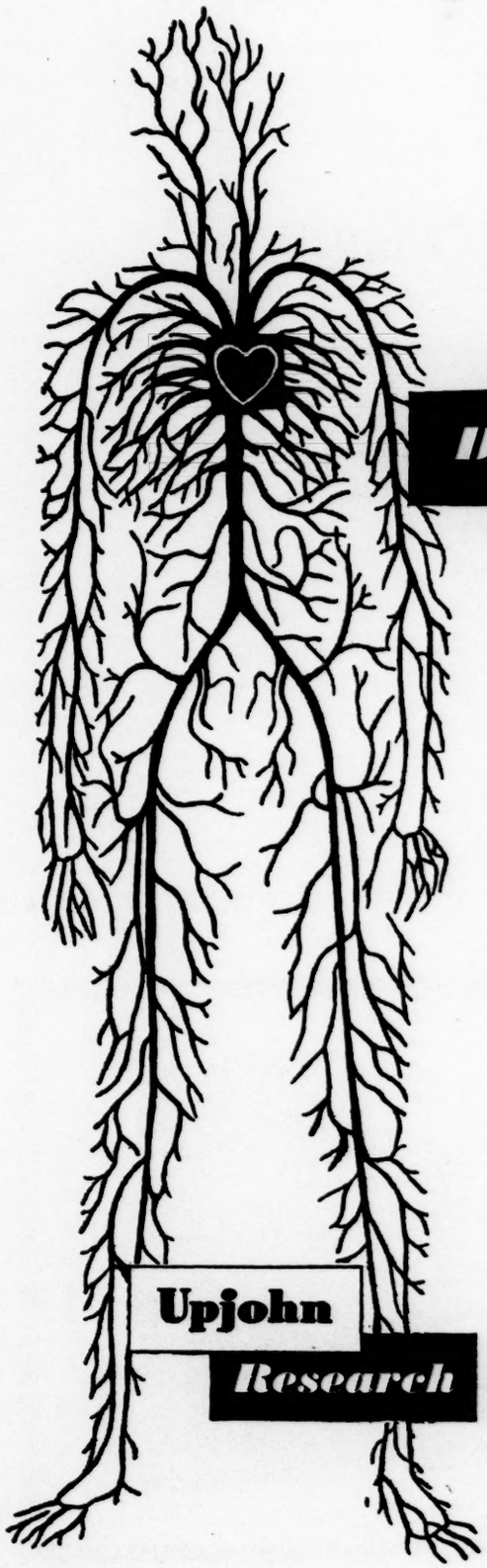

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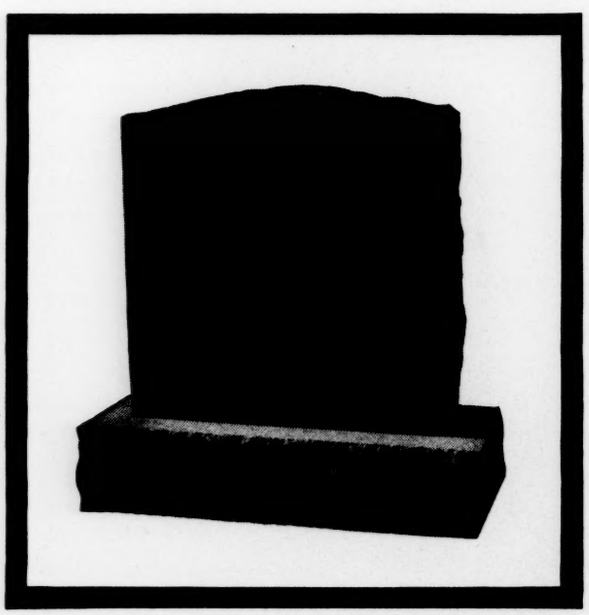

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Hewitt, W. L., and Williams, B., Jr.: New England J. Med. *242*:119, 1950

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Payne, E. H.; Knaudt, J. A., and Palacios, S.: J. Trop. Med. & Hyg. *51*:68, 1948

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Ley, H. L., Jr.; Smadel, J. E., and Crocker, T.: Proc. Soc. Exper. Biol. & Med. *68*:9, 1948

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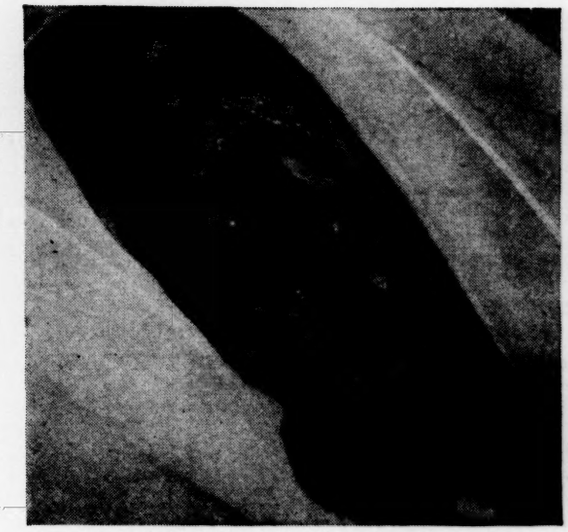

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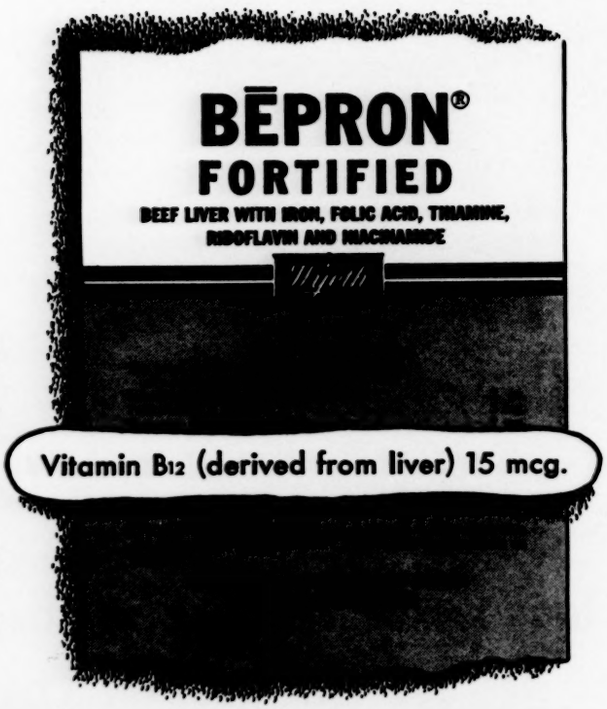
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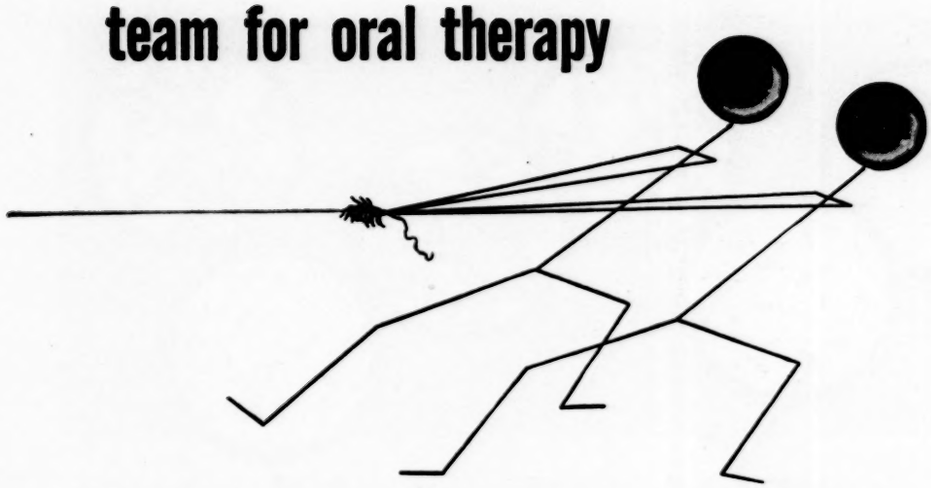

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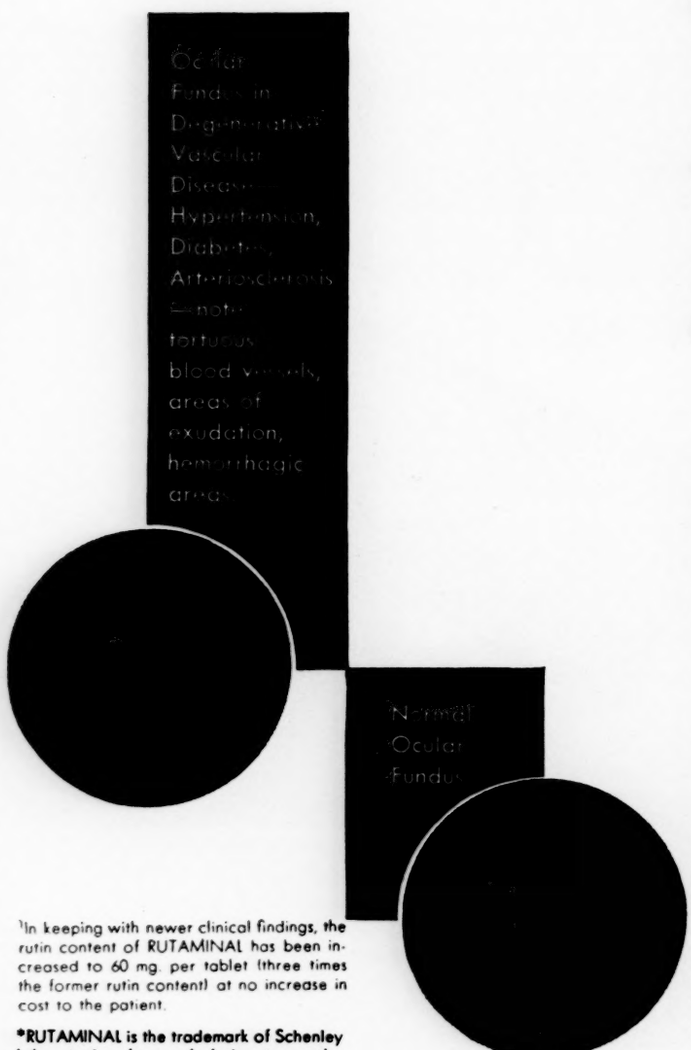

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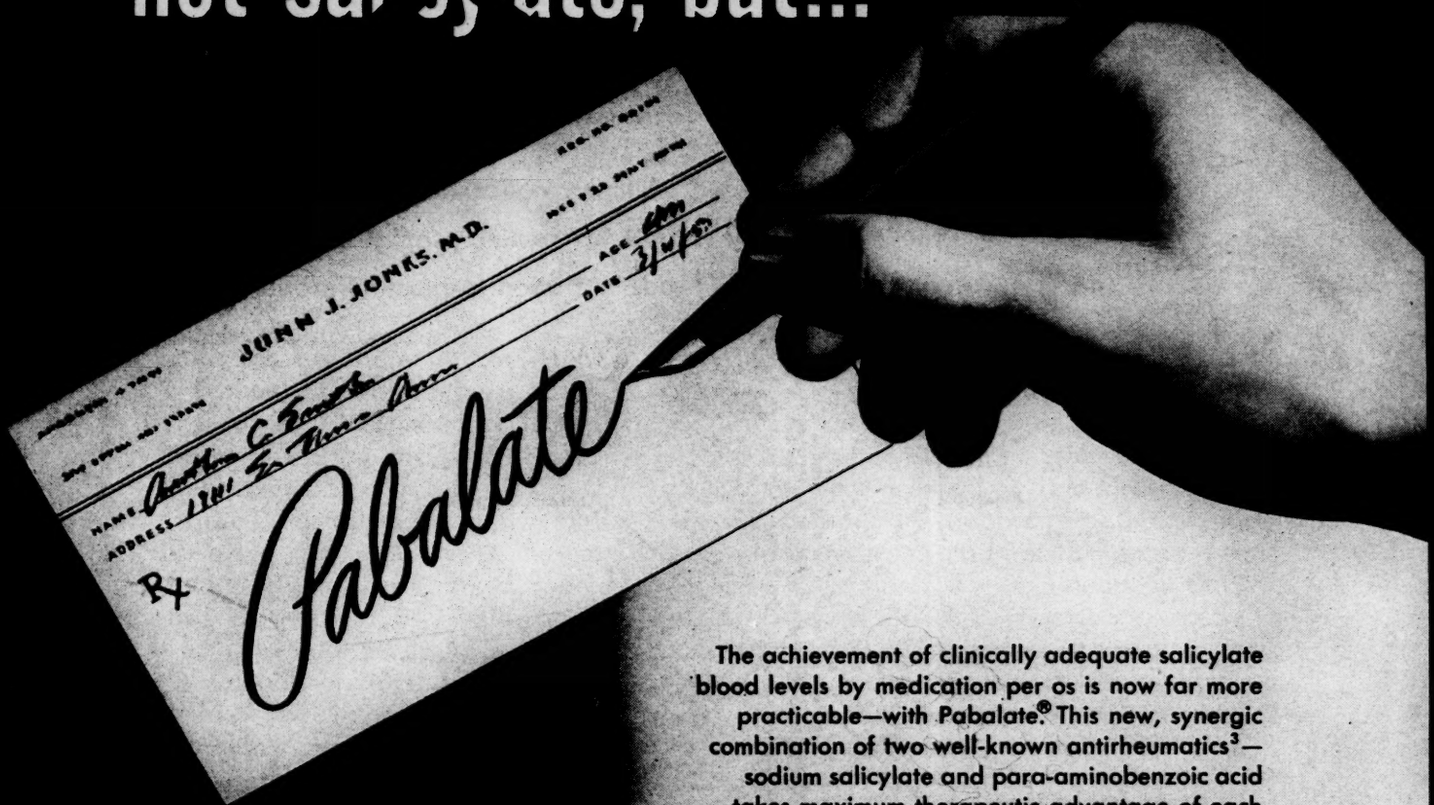

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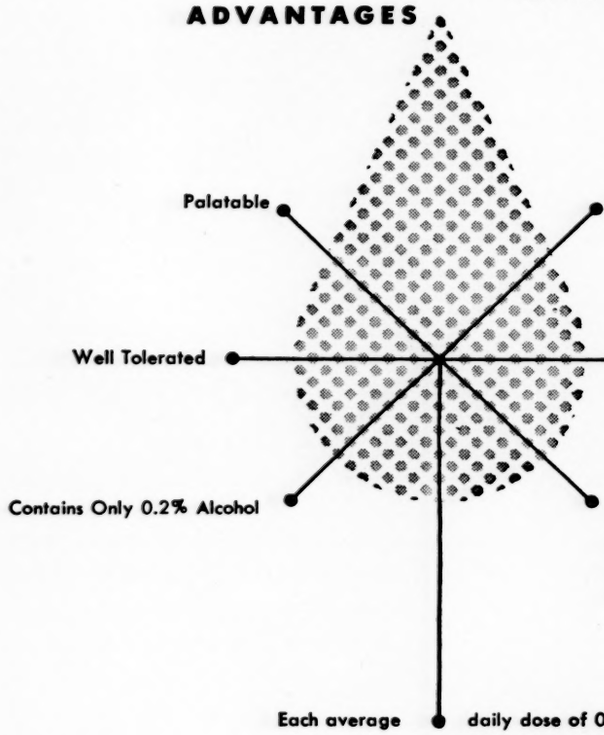

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The American Journal of Medicine

VOL. VIIII MAY, 1950 No. 5

Foreword

WRITTEN testimony of the interest aroused by the hundreds of thousands of cases of viral hepatitis which occurred during the past decade is afforded by the listing of 775 papers on this subject in the Quarterly Cumulative Index Medicus during the period from January, 1940, through June, 1948. With such an abundance of writing it would not be unusual for critics to question the need for a symposium on hepatitis. However, it is believed that the information contained in the following papers, particularly as it relates to epidemiologic and clinical matters, is of importance in complementing the knowledge now available on the natural history of this disease.

The purpose of the Symposium is to describe certain experiences of the American Occupation Forces in Germany with viral hepatitis. In the two years after cessation of hostilities in Germany the incidence of this disease among American troops steadily increased to the point where it again assumed proportions comparable to those of 1945. The magnitude of the problem prompted establishment of a Hepatitis Center at the 120th Station Hospital in Bayreuth for the treatment of all patients with the disease. In addition to its value as a therapeutic center the hospital provided the following opportunities: (1) to study the clinical course of disease; (2) to extend and elaborate existing knowledge about certain tests of hepatic function; (3) to make epidemiologic observations and (4) to serve as a source of acute and convalescent phase materials for study in the United States. Working conditions in Germany during this period were not adapted to studies on the virus of hepatitis.

Approximately 4,000 patients with viral hepatitis were treated at the Center. By reason of the large number of patients and the completeness of study of various aspects of hepatic function, a vast amount of data was accumulated. It is not the purpose, however, of this Symposium to report on all such material but rather to select certain data which illustrate new observations or extend and elaborate previous studies of others. Thus variations in the amounts of serum alkaline phosphatase and cholesterol, and certain of the factors influencing the retention of bromsulfalein in the blood have been investigated. Further correlation of the results of various flocculation tests has been made, and the capacity of the liver of such patients to excrete radio-opaque dye in sufficient quantity to allow visualization of the gallbladder by roentgenogram has been studied. Of interest also is the adaptation of a test of hepatic function as an epidemiologic tool by using bilirubinuria as an early sign of hepatitis in men exposed to the disease.

Much has been written about the clinical course of the disease, and possible modes of its spread and means of prevention. A number of clinical descriptions have emphasized the severity of the disease, the dangers of residual hepatic damage, and the necessity for prolonged rest in bed and excellent diet during convalescence. These concepts were largely derived from the experience of treating men exhausted by

wounds and privation. In contrast to these previous observations Dr. Swift and his colleagues have described the mildness of hepatitis in Germany during the past two years, suggesting that the more rigid regimens of therapy formerly advised might be modified in previously healthy young adults when certain criteria were observed.

Many questions concerning the epidemiology of viral hepatitis remain unanswered. Although possible methods of spread of both epidemic hepatitis and homologous serum hepatitis are known, their interrelationships and the conditions which influence alterations in their incidence are often poorly defined. In this regard the experience which is recorded in the papers on the history and on the endemiology of hepatitis in Germany is of interest. Dr. Paul and Dr. Gardner have documented the available records of this disease in the indigenous population as well as in American troops, and have discussed the possible effects of the introduction of young, susceptible men into an area where the disease has apparently been diffusely spread for many years. They have also indicated the complexity of the relationship between the *naturally* and the *artificially* transmitted forms of hepatitis in American troops in Germany, and have suggested that a larger percentage of infections than previously believed were artificially transmitted. The importance of this in regard to possible methods of control is evident.

The work to be described here represents the experience of two years, 1947–1949, and marks, as it were, the end of one phase of the original program. In May, 1949, the Hepatitis Center was transferred to the 98th General Hospital in Munich where it has been possible to develop other interests in the study of the disease and, during the past year, Dr. Dorothy M. Horstmann has been conducting investigations primarily concerned with hepatitis virus. A number of clinical investigations, including study of the effect of newer antibiotics and correlation of results of tests of hepatic function and histologic alterations of the liver, are being carried on but increasing emphasis is now being given to studies of virologic and epidemiologic nature.

W. Paul Havens, Jr., M.D.
Jefferson Medical College,
Philadelphia, Pa.

Symposium on Viral Hepatitis

A Note on the History of Epidemic Viral Hepatitis in Germany*

HORACE T. GARDNER, M.D.
New Haven, Connecticut

THE history of hepatitis in Germany provides documentary evidence of an extensive experience with this disease, and the records indicate the importance of German physicians as contributors to the knowledge of the subject. It is evident that in reviewing such a history caution must be exercised in distinguishing between viral hepatitis and other febrile diseases associated with jaundice. The frequency of the occurrence of leptospirosis in Germany and the possibility that yellow fever might, at some time, have spread from Spain require consideration. The appearance of jaundice in malaria and occasionally in other infectious diseases must also be taken into account. In addition, it is possible that diseases now unknown may not only have changed character but may have disappeared altogether.

The first reference to a contagious or infectious type of jaundice in Germany (and, for that matter, in Western Europe) was mentioned by Ford[1] as occurring in a letter written by the Pope, St. Zacharias, in 751 to St. Boniface, Archbishop of Mainz and Primate of Germany. One cannot help but ascribe considerable importance to a disease which won recognition in such high places. Although jaundice associated with stone in the common bile duct was described, no further reference to icterus in epidemic form in Germany has been found from that time until the turn of the eighteenth century when there were casual and incomplete accounts of jaundice occurring in Berlin (1699 and 1702).[2] Subsequently, recognition of the disease apparently became more generalized and, in 1751, Cleghorn[3] commented on the occurrence of what was probably infectious hepatitis on the Island of Minorca; this was later cited by Cockayne[4] as the earliest described epidemic of this disease. The first paper which was wholly devoted to an outbreak of infectious hepatitis appeared in 1761[5] and described a small epidemic which reached its peak in the winter. According to von Bormann[6] the clinical description did not differ from that of mild acute infectious hepatitis as it is currently known, with slight fever lasting one to two weeks, followed by scleral icterus, dark urine and a feeling of fullness or discomfort in the right hypochondrium. During the latter half of the eighteenth century the military importance of this disease was evident, and descriptions of epidemic bilious fevers[7,8] occurring in British troops in Germany undoubtedly included what is now known as epidemic hepatitis.

In the nineteenth century epidemics of viral hepatitis in Germany apparently were common. In many of them juvenile populations were largely involved, suggesting the probability that hepatitis was becoming established as an endemic disease. Some of these outbreaks are of particular interest in the light of more recently acquired knowledge. Thus Mende[9] observed that in an

* From the Section of Preventive Medicine, Yale University School of Medicine, and the 120th Station Hospital (Hepatitis Research Team), European Command, U. S. Army of Occupation in Germany. Representing work done under the auspices of the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, Office of The Surgeon General, U. S. Army, Washington, D. C.

epidemic at Greifswald in 1807–1808, only about 25 per cent of the sick actually developed jaundice but he considered the remainder to be affected with the same disease, anticipating Eppinger's[10] later, more accurate description of anicteric hepatitis. Stitzer[11] in 1876 studied an outbreak confined to the women of a family in Mainz. On investigation it was determined that the only contact which the five females of the household had which the men did not have was in the kitchen, and here a drain was found which was contaminated with feces. Interest in the pathogenesis of hepatitis found expression during this period and a suggestion of the possible identity of "catarrhal jaundice," epidemic hepatitis and certain cases of acute yellow atrophy as different manifestations of the same disease was made by Fröhlich[12] in 1879. It now appears that viral hepatitis must have been very common in Germany during the past eighty years, more particularly in the past two decades. Bachmann[13] listed chronologically sixty-four epidemics of the disease among civilians in Germany during this time, more than half of which occurred since 1935. These epidemics have apparently been widespread, covering the whole country, and between epidemics moderately high endemic rates have prevailed with evidence of greater incidence of disease in Bavaria than in certain other regions.

In addition to the contributions made to the knowledge of epidemic hepatitis, the first recognized description of what is now known as homologous serum hepatitis was made in 1885 by Lürman.[14] During a period of nineteen days 1,289 workmen in a shipbuilding yard in Bremen were vaccinated against small pox with human glycerinized lymph. Within a few weeks to several months 191 of these men became jaundiced, while 500 men vaccinated with *different* lymph at the same time did not acquire jaundice. In the city of Bremen during the same period there were only a very few sporadic cases. Lürman concluded that the outbreak was due to "bad lymph" and commented on the unusually prolonged incubation period of several weeks. A similar report appeared the same year from Merzig am Saar by Jehn[15] who described Jennerian vaccination of five groups of patients by five different doctors using lymphs from different sources. The incubation period of the hepatitis which subsequently developed in certain groups ranged from two to eight months. The numbers vaccinated and the numbers becoming sick were recorded as follows:

POSTVACCINAL HEPATITIS IN 1885

No. Vaccinated	No. Who Developed Hepatitis
540	141
446	35
243	14
50	1
87	0

EPIDEMICS AMONG TROOPS

In the eighteenth and nineteenth centuries infectious hepatitis was a disease of military importance among German and other European troops in Germany and France, both in the field and in garrison.[12,16] The French referred to the disease as jaunisse des camps, and in Germany it came to be known as Kriegsikterus or Soldatengelbsucht. During and after World War I there was a great increase of epidemic hepatitis as well as of homologous serum hepatitis in Germany, and the experience in the Navy and Army was recorded by Ruge.[17] It was in this period that Eppinger[10] suggested that the pathogenesis of the disease was primarily concerned with necrosis of the hepatic parenchymal cells rather than obstruction of the common bile duct by a plug of mucus, as previously defined by Virchow.[18] In Germany as elsewhere however, physicians were slow to accept this new concept. After 1926 the incidence of viral hepatitis apparently diminished in Germany, as in other countries, followed by a gradually increasing number of cases both in civilians and troops after 1933.[19–21]

During World War II hepatitis was a scourge to the German Army on almost all fronts. There were outbreaks among German troops in Norway in the early spring

of 1941[22] and in the German Army occupying France. In July, 1942, large epidemics occurred among German troops in Greece, in the Afrika Korps in Africa[23,24] and on the Eastern front[25] reaching, according to Gutzeit,[26] peaks of highest incidence in Greece in August, in Africa in September and on the Russian front in October. There is no doubt that hepatitis must have been common among German troops in Russian prison camps, as many men coming back to Germany in 1947–1948 gave histories of such outbreaks and similar reports were obtained from repatriated Germans in 1948–1949.[27] The great importance of hepatitis as a military disease stimulated numerous investigations in various parts of the world, and it is of interest that Voegt[28] in Germany was the first to demonstrate in volunteers the infectivity of the duodenal contents and blood of patients with the disease.

While most of the records available from Germany during the past decade came from military sources, there were also occasional contemporary reports of hepatitis in the civil population. In the autumn of 1942 a large outbreak occurred near Leipzig,[29] and Bachmann[13] listed some ten University Hospitals or Clinics which recorded an increased number of admissions since 1939. However, it is impossible to determine the exact or even the relative incidence of viral hepatitis among the indigenous population of Germany both before and after World War II. The disease was made reportable in the American Zone of Occupation late in 1947 but, in general, reporting has been unreliable so that the civilian statistics available are not of great value.[30] Nevertheless, unofficial information from local physicians and Public Health authorities make it quite clear that the disease is widespread and occurs far more frequently than is reported. The possible relationship of this widely diffused, endemic disease in the German population to the high incidence of hepatitis in the American Army of Occupation is discussed in the next paper.[31]

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Endemiologic Aspects of Hepatitis in U. S. Troops in Germany 1946–1950*

JOHN R. PAUL, M.D. *and* HORACE T. GARDNER, M.D.
New Haven, Connecticut

ACUTE hepatitis, which was prevalent among troops in Europe during the recent war, continues to be a disease of high incidence among U. S. troops stationed in Germany during the postwar period (1946–1950). The morbidity rates have been higher than in U. S. troops stationed elsewhere. A glance at Figure 1 tells this story more graphically than a brief description could do. It has been our purpose, consequently, to review the epidemiologic circumstances under which this situation has been taking place in an attempt to interpret it in the light of current theories regarding modes of transmission of viral hepatitis. In particular, we have been concerned with an analysis of the circumstances under which the high rates for hepatitis have prevailed. We realize from the outset, however, that we have not come to grips with this situation, for there is so much that is unknown about the epidemiology of viral hepatitis and so little, as yet, which can be done to control the disease.

This recorded high incidence of hepatitis occurring in the U. S. military populations in Germany is hard to explain and there may be at least two forms of disease involved: so-called infectious hepatitis (IH) and serum hepatitis (SH). Although the clinical pictures of IH and SH are practically indistinguishable, there is evidence that the two viruses which cause these conditions differ and that their mode of transmission may sometimes differ. Not the least of such differences is the fact that the virus of SH is presumably non-infectious by mouth, and not recoverable from the feces of patients—properties which stand in some contrast to those of IH virus. Furthermore, the virus of SH is apparently able to maintain itself as a viremia in the blood stream for long periods of time without causing obvious illness and may, therefore, persist in a symbiotic relationship[1,2] with its host more readily than seems to be the case with the virus of IH. Nevertheless, these two forms of hepatitis are apparently members of the same family, and some observers[3,4] have claimed that too much is being made of the differences between IH and SH, suggesting that the latter is a "modified type" of the former, depending upon reactions of the host. To support this general relationship we have the fact that acute hepatitis and serum hepatitis have been found together within the same area, as was the case in North Africa in 1943[1] and in Italy later. Consequently, we shall continue to assume that the viruses of IH and SH are probably members of the same family, although one should be aware of certain inexplicable differences which seem to exist between the diseases caused by these respective agents and which complicate the epidemiologic picture.

THEORIES OF TRANSMISSION

The theories of transmission will be reviewed but briefly here. For more extensive considerations, the reader is referred to the recent article by Havens.[5]

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Infectious Hepatitis. Most epidemiologic evidence indicates that infectious hepatitis is spread through some form of person-to-person contact, although explosive water-borne and food-borne epidemics have been described. The exact manner in which this

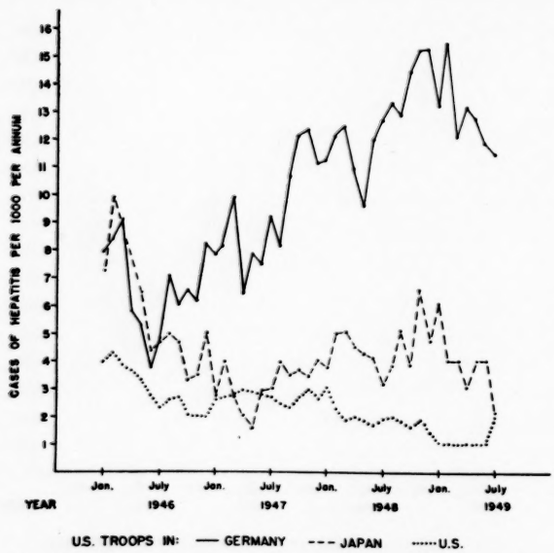


FIG. 1. Case rates for hepatitis in U. S. troops stationed in Germany, Japan and the United States during the three and a half-year postwar period, 1946–1949.

contact infection occurs has not been established beyond doubt. There is indeed good experimental evidence to indicate that the *intestinal-oral circuit* plays a part in the natural person-to-person spread. This concept is also based in part on military observations that the disease prevails best when and where the sanitation of camps is poor. It receives further support from civilian experiences such as the frequent outbreaks of hepatitis encountered in institutions for mentally deficient individuals, in which the rates have been higher in those buildings housing individuals with the lowest mental capacity and, therefore, in which the poorest sanitation can be maintained. On the other hand, Gauld[6–9] has considered the possibility of *respiratory spread* which may be more consistent with the seasonal winter pattern of prevalence of hepatitis. But experimental evidence of this is not satisfactory since attempts to recover the virus from the naso-pharynx in the active stage of the disease have nearly always been unsuccessful. The possibility of transmission by a biting insect such as the louse, bed bug or mosquito, deserves consideration but this seems to be remote.

Nevertheless, regardless of the orifice or surface area of the human body from which infectious material escapes, nearly all investigators agree that personal contact is the *usual* method of transmission. But over and above this the *artificial* spread by means of syringes or needles of the virus of infectious hepatitis (IH) with its shorter incubation period of sixteen to thirty days, as well as the artificial spread of the virus of serum hepatitis (SH) with its long incubation period of 50 to 150 days is also an established fact. The hardiness of the causative agent (or agents), the infectiousness of minute quantities of blood and the increasing use of inoculations suggest that the two forms of hepatitis probably have been induced artificially and unintentionally more often than is realized.

Serum Hepatitis. According to our limited knowledge, this disease originates in a mysterious manner but once the viremia has been established the only known means of spread is artificial either through the inoculation of a product of human blood or by the use of an instrument which had been contaminated by human blood containing the virus of SH.* Whether SH is a variant of IH for which the syringe serves as an efficient mechanical vector, one cannot

* There is a long list of circumstances under which the artificial transfer of viral hepatitides may occur, including the use of transfusions, inoculations of serum and a variety of procedures which involve the use of syringes or needles which have not been properly sterilized between inoculations.[11] Such happenings have occurred in diabetic clinics, arthritic clinics, in clinics for the inoculation of arsenicals and antibiotics. One might further speculate on other means of transmission such as the inoculation of local anesthetics in the course of dental extractions, and during inoculations of various antigens and vaccines for the immunization of military personnel. The Medical Department of the Army has been cognizant of various means of transmission of viral hepatitides and Directives have been prepared and issued to control the situation.[12] So far, the military rate for hepatitis among American troops in Germany does not seem to have been influenced by any of the precautionary measures taken.

say but it is safe to say that the virus of SH did not originate in a syringe.

The long incubation period characteristic of at least certain strains of SH may vary tremendously. For instance, Parr[10] has described an outbreak of serum hepatitis which occurred at Camp Polk in the United States in 1942. On February 27th over 5,000 soldiers were inoculated with a yellow fever vaccine containing icterogenic human serum. As a result, 1,004 cases of serum hepatitis developed at various intervals among these soldiers, all of whom had been injected on the same day with the same dose of the same lot of vaccine. Figure 2, taken from Parr, illustrates the variability of the incubation period in these cases. It ranged from 63 to 137 days, and the daily case incidence curve is not unlike that of a protracted epidemic in which the agent is spread from person to person. This is illustrative of some of the complexities which arise in tracing the epidemiology of serum hepatitis.

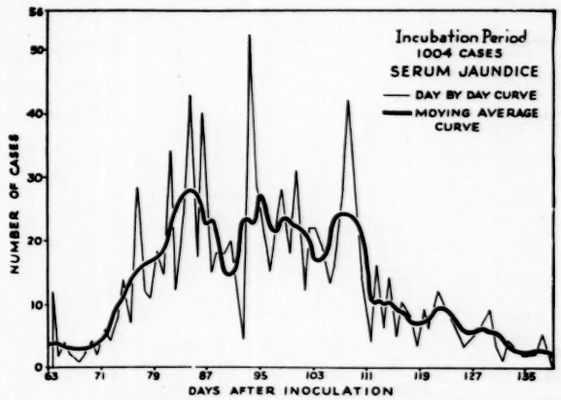


FIG. 2. Variations in incubation period of serum hepatitis (SH) as recorded in 1,004 persons inoculated simultaneously with the same dose of the same lot of material. Data from Parr.[10] Illustration previously reproduced by Horstmann and Paul in *J. A. M. A.*, 135: 11, 1947.

GENERAL ASPECTS OF THE EPIDEMIOLOGY OF HEPATITIS

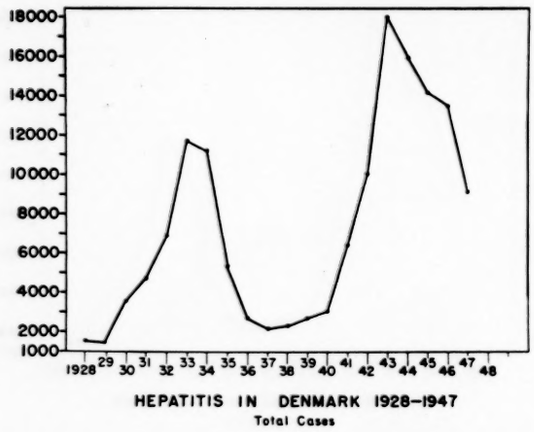
Often the reporting of civilian cases of infectious hepatitis has in the past been a hit-or-miss affair so that accurate figures on its civilian prevalence are quite limited. One reason which has contributed to the inaccuracy of the reporting is that most of the civilian cases occur in children. Inasmuch as acute hepatitis is a disease which is apt to be milder in children than in adults,[13] the juvenile cases are often regarded as trivial and not worth treating, let alone reporting. Consequently, it is not remarkable that accurate civilian data on the endemic and even epidemic incidence of hepatitis have not readily found their way into vital statistics. Sweden and Denmark have been exceptions to the general rule. The disease has been common and notifiable in those countries since 1928 and excellent data are available. (Figs. 3 and 4.)

Although infectious hepatitis is geographically widespread and probably occurs throughout the world, there are certain areas with an established record of high endemic prevalence of which the American Zone in Germany is one. (Fig. 5.) Another, in particular, is the Mediterranean littoral, and severe epidemics have occurred repeatedly among foreign troops who have been brought into that general area. This was the common experience of both World Wars I and II. But infectious hepatitis is not limited geographically or from the standpoint of climate to any special part of the world, although it is believed to flourish best where sanitary conditions are poor. High rates for U. S. troops during World War II were encountered in China and the Philippines.

Seasonal fluctuations in the prevalence of infectious hepatitis have been observed in many different parts of the world. The characteristic trend is for epidemics to occur in the autumn or early winter months, with a decline in incidence during mid-winter. This has been the common experience almost everywhere. No truly adequate explanation for this seasonal trend has ever been put forward. The best that can be said is that it may result from the crowding which takes place at the onset of cold weather, with the idea that closer personal contact may account for the higher incidence. In some places hepatitis, however, is

said to occur constantly throughout the year with little apparent seasonal variation.

From the standpoint of the *age* group most liable to be attacked, infectious hepatitis is primarily a disease of childhood. The experience of most civilian outbreaks records 65 per cent or more of the cases in children.[13] There is a relative dearth of age incidence statistics from civilian populations, but in England Lisney[14] reports that among 3,800 inhabitants of East Suffolk the attack rate for the school children was 54.5 per 1,000, as opposed to a rate of 6 per 1,000 for the total population within the same community. The highest attack rate occurs in the age group of ten to fourteen years.[15] But young adults up to thirty years of age are also quite susceptible as indicated by the high prevalence of the disease among troops, to which frequent reference will be made subsequently in this article. Above the age of thirty, however, the incidence of disease falls off rather definitely. It has not been determined whether immunologic or constitutional factors or risk of exposure have been responsible for the adult-sparing effect.



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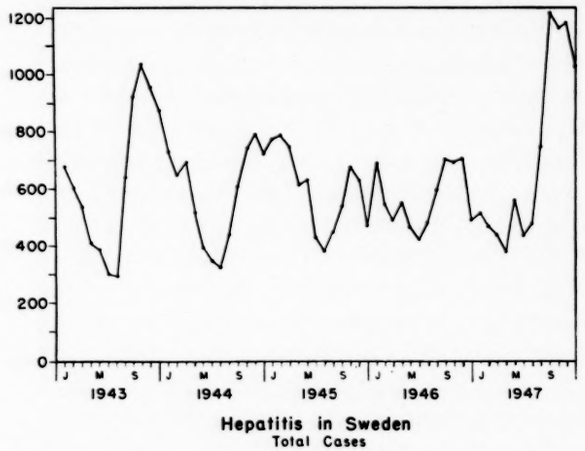

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FIG. 3. Cases of hepatitis in Denmark during the past twenty years.
FIG. 4. Civilian hepatitis cases in Sweden (1943–1947) showing seasonal fluctuations in the number of cases.

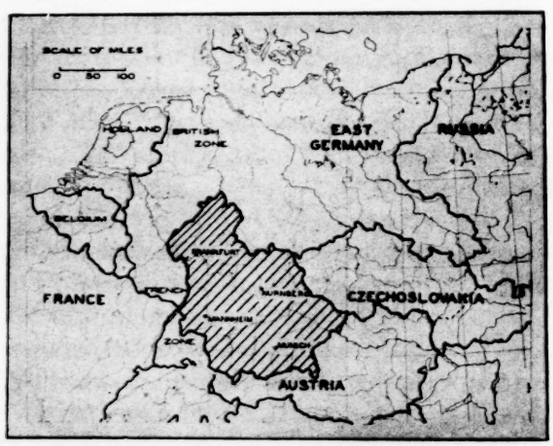


FIG. 5. Map of German territory, 1949. The shaded area indicates the U. S. Zone of Occupation. The entire southeastern half of this zone lies in Bavaria.

Epidemics may be explosive or protracted. The usual civilian outbreak is prolonged and may involve hundreds of cases during the course of two to three months. Family and institutional outbreaks are common; in the former it is usual for hepatitis to appear first in one member of the family and then be followed in two to four weeks by subsequent cases.[16] Institutional epidemics, particularly in schools and asylums, may involve as many as 80 per cent of the population.

In the military history of this disease the pattern is usually that of a slow spread through a given unit covering a period of three to four months. Sometimes there are variations in incidence of the disease among special military groups. Thus British officers in the Middle East and Sicily (1942–1944) had attack rates 4.7 times as great as those in other ranks.[17] Gauld reported a somewhat similar situation among certain groups of American troops.[8] Attempts to explain

the higher incidence in officers on the basis of environmental factors have pointed to the fact that enlisted men used individual mess kits and were responsible for their cleanliness whereas the officers messed together and were exposed to a greater possibility of contamination of their food by carriers or individuals suffering from the subclinical disease who worked as food handlers. Environmental factors have also been invoked to explain the significantly higher incidence of hepatitis in certain epidemics among the headquarters personnel as compared to combat troops.[8] The former are usually subject to more crowding and person-to-person contact is more common.

In summary, therefore, the bulk of epidemiologic evidence at present suggests that viral hepatitides flourish particularly well in certain parts of the world, usually in the autumn and early winter, and that the spread of these diseases is facilitated not only by such factors as crowding and poor sanitation but perhaps also by the transfer of the infection via needles.

U. S. MILITARY EXPERIENCE WITH HEPATITIS IN EUROPE AND NORTH AFRICA DURING WORLD WAR II

Hepatitis was a veritable plague among British[17] and U. S. troops in North Africa, Sicily and Italy in 1943–1945[6–9] for the rates in the latter were very high. (Fig. 6.) The story of this disease in the Mediterranean area has been well documented from an epidemiologic point of view by Gauld[6–9] and from a clinical point of view by Havens[18] and by Barker et al.[19]

Beginning in August, 1943, at approximately the time when the Allied troops crossed the Mediterranean Sea to invade Sicily, the military rates for hepatitis in U. S. troops began to rise sharply and for the next two years exceedingly high winter rates were maintained in this area. Sometimes as many as 35 or 40 per cent of certain military units were invalided over a three-month period.

In keeping with the usual seasonal trend, an autumnal and early winter prevalence was the experience of U. S. troops at this time in the Middle East, North Africa and Italy;* and one can actually speak of these military epidemics of hepatitis in the Mediterranean littoral as occurring periodically

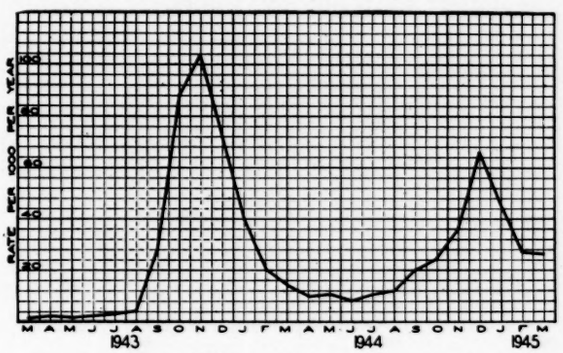


FIG. 6. The high and fluctuating rates (per 1,000 per annum) at which U. S. troops contracted hepatitis in the Mediterranean area during the combat years of 1943–1945. From Gauld.[6]

in the form of *autumnal* outbreaks which often were prolonged into the winter season. The sharp rise in these areas usually began in September, a peak was reached in November or December and the decline occurred rapidly in January.

These epidemics occurred in the presence of poor sanitary conditions and some of the outbreaks in Italy are believed to have resulted from the drinking of contaminated well water.[7] Most epidemiologic evidence indicated, however, that the usual spread of the infection was the result of some form of person-to-person contact which presupposes a high rate of infection among the local civilians. Exact data on this local civilian prevalence of infectious hepatitis were not available in 1943–1945 but the indications were that mild hepatitis was endemic and common there although largely unrecognized.

In the Mediterranean area the rate at which fresh troops acquired the disease was much higher than that of troops seasoned by a year of exposure. And in this respect it is pertinent to point out that there was a

* According to Gauld[6] the peak in both British and German troops in Italy in 1944 occurred about two months earlier than in the American forces.

large number of soldiers in the Sicilian and Italian invasion force of 1943 who had contracted serum hepatitis (SH) in the previous year, 1942; but according to Gauld's analyses[9] they were not protected by this experience. Indeed, the IH rate among them was higher than among others.

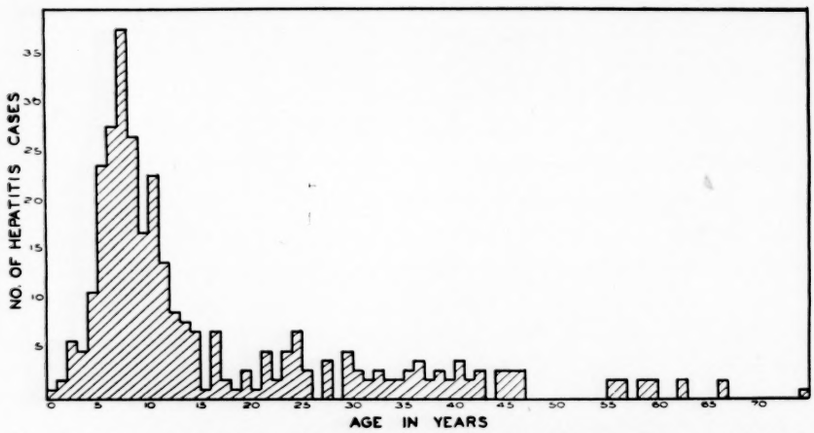


FIG. 7. The age incidence of hepatitis epidemica in Germany prior to World War II. The data are from two outbreaks occurring in Hamburg and Wilhelmsberg, respectively, described by Holm in 1939, and quoted from von Bormann.[22] The preponderance of juvenile cases is apparent.

This drastic experience with hepatitis in Italy during the combat years of the War, 1943–1945, was repeated later for U. S. troops in combat in France and Germany in the fall and winter of 1944–1945, but the *very high* rates which had prevailed in Italy during the previous winter were not encountered in France and Germany. This was the prelude to the postwar experience with hepatitis in U. S. troops in Germany.

HISTORY OF VIRAL HEPATITIS IN GERMANY

In another article in this series[20] mention is made of the fact that Germany is practically the home of viral hepatitides as far as North Europe is concerned. The story of *hepatitis epidemica* in Germany goes back some two hundred years to an epidemic reported by von Herlitz in Göttingen in 1761. It has been almost impossible to determine until relatively recent years how many of the epidemics described were examples of the disease as we now know it but, apparently, such epidemics have been common. Bachman[21] lists chronologically sixty-four epidemics designated as "infectious hepatitis" reported among civilians in Germany during the past eighty years, more than half of which occurred since 1935. These epidemics have apparently moved back and forth across Germany and for the past forty years the disease has been considered to be endemic throughout the whole German territory, rural and urban locations alike. Bachmann believes that it was after World War I, and particularly during the period 1920 to 1937, that hepatitis became heavily seeded in the German population but it must have been very common before that period also. Among civilians there, it has largely been a children's disease. At least this is true of recent epidemics if we can judge from Holm's figures (1939) (quoted by von Bormann[22]). (Fig. 7.) Two-thirds of these cases were below the age of sixteen. This low age incidence bespeaks the local endemicity of this infection.

In the *German Army*, both in the garrison and in the field, hepatitis has also long been a scourge. Military epidemics have been reported over the past hundred years or more, of which, since 1869, Bachmann lists thirteen occurring within Germany and as many outside of Germany. During World War II the German Army suffered severely from epidemic hepatitis.[21,23,24]

Annual rates for jaundice in the German

Army and Navy in Germany during the 1920's, taken from Ruge's article,[25] appear in Table 1. The Navy rates are broken down to include so-called postarsphenamine or salvarsan jaundice, and catarrhal jaundice.

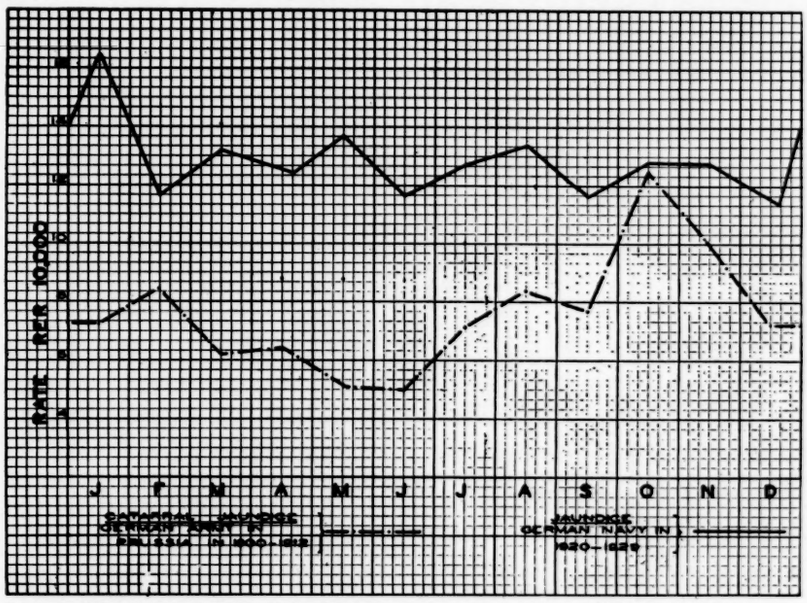


FIG. 8. Military data from the German army and navy which indicate that in the army, in which cases were largely "catarrhal icterus" or "hepatitis epidemica," the peak occurred in October, whereas in the navy a large fraction of the cases were *post-salvarsan icterus* and the peak occurred in January. After Ruge.[25]

Mention has already been made of the fact that Bavaria was a favored site in Germany for hepatitis. During the period 1900–1912 the hepatitis rate for the Imperial Bavarian Army was higher than that of the Imperial Prussian Army. These somewhat ancient German Army hepatitis rates of two decades ago, listed in Table 1, prove to be but one-tenth as high as those encountered among U. S. troops in Germany today; but it should be appreciated that the German rates represent soldiers and sailors quartered on their own home ground. Had these men been quartered in foreign areas the story might have been different. In fact, it was different in the sailors of the German Navy who were on sea duty, for their jaundice rates were from 50 to 150 per cent higher than those on shore.

TABLE 1
JAUNDICE RATES PER 1,000 IN THE GERMAN ARMY AND NAVY (ON SHORE DUTY) IN GERMANY (FROM RUGE)[25]

Year	Army	Navy		
		Jaundice (All Types)	Catarrhal Jaundice	Jaundice after Salvarsan
1922	0.82	1.48	1.21	0.23
1923	....	1.80	1.27	0.51
1924	0.85	1.45	0.91	0.46
1925	0.72	1.14	0.88	0.26
1926	0.66	0.82	0.72	0.10
1927	0.55	1.01	0.82	0.16
1928	0.49	0.58	0.42	0.13
1929	0.56	0.50	0.36	0.12

Syringe hepatitis is not new to Germany, either. At least it received attention during the 1920's in individuals who were being treated for syphilis (Salvarsan Gelbsucht). During the 1920's Ruge's studies[25] of jaundice occurring in naval personnel receiving antisyphilitic treatment led him to the conclusion that idiopathic catarrhal jaundice (*einfache katarrhalische Ikterus*) and jaundice occurring in patients receiving antisyphilitic therapy (Salvarsan Gelbsucht) were one and the same infectious disease. In this observation he had been anticipated by some ten

years by Dr. John Stokes and his colleagues[26] at the Mayo Clinic in this country, who in 1920 reported some seventy cases of postarsphenamine jaundice and presented strong circumstantial evidence that an infectious agent, presumably the same as that

TABLE II
HEPATITIS RATES PER 10,000 IN GERMAN CIVILIANS IN U. S. ZONE

Month	1946	1947	1948	1949
Jan	0.6	1.4	2.5	1.3
Feb	1.9	1.3	2.6	1.6
March	1.7	1.6	1.7	1.1
April	0.4	1.6	1.4	0.8
May	0.4	1.1	1.3	1.2
June	0.2	1.1	1.4	1.3
July	0.2	0.8	1.2	1.0
Aug	0.3	1.5	1.7	1.2
Sept	0.3	2.0	1.8	1.4
Oct	0.5	2.8	1.2	...
Nov	0.6	2.1	1.4	...
Dec	0.3	2.2	1.2	...

which caused epidemic infectious jaundice, was the cause. Ruge charted the incidence in the German Navy of postarsphenamine jaundice and catarrhal jaundice month-by-month over a period of years and noted that the peak for catarrhal jaundice in the Navy as well as in the German Army was in October, whereas the postarsphenamine jaundice peak occurred in January, namely, three months later. It is tempting to speculate on the reasons for this time relationship. (Fig. 8.) We will not do so at this point but shall discuss this matter later in this article.

It would be highly desirable, of course, to have accurate records of the *postwar* prevalence of hepatitis of any type among German civilians throughout the American Zone but these data are not easily obtained, for the epidemic disease, in contradistinction to the sporadic disease, was the only type reported there until 1947 when reporting of all types was made compulsory.

For what they are worth, however, civilian rates for "hepatitis" covering the period from January, 1946, through September, 1949, are recorded in Table II. The rates are low—due to inadequate reporting, no doubt, but in part perhaps to the preponderance of juvenile and therefore mild cases, many of which are so mild as to deserve little or no medical notice. The only reported recent, postwar civilian "*epidemic*" of hepatitis in Germany which has been listed in the American Zone occurred in Hanau.[21] There was a corresponding rise in the rate among U. S. troops quartered in that city during the summer of 1948.[27]

RECENT CIVILIAN HEPATITIS RATES IN COUNTRIES ADJACENT TO THE AMERICAN ZONE

As an indication of contemporary prevalence of hepatitis in Europe the rates from several countries, including Switzerland, Denmark and Sweden, have been reviewed. They all indicate that during recent years hepatitis has been common in those countries also. Fortunately, the records from the Scandinavian countries are unusually accurate. In Denmark the experience has been rather special, recording a sudden rise in the hepatitis rate at the time of occupation of their country in 1940 by the German Army, with subsequent persistence at a rather high rate. (Fig. 3.) But the picture in Denmark has been somewhat confused by the presence of a severe form of hepatitis which affected middle-aged women in particular. It was prevalent during the period 1946–1947.[28] It is unknown whether this disease is a variant example of viral hepatitis or not. It also has been reported from Switzerland.[29]

In Sweden, too, (1943–1947) hepatitis has been endemic but the rates have been lower than those recorded in Denmark. (Fig. 4.) In all of these European countries there has been the characteristic seasonal swing, with cases increasing in the fall and subsequently declining either early or late in the winter. The record from Sweden, charted in Figure 4, is a good example of this. And when the experience of several years is pooled from Sweden and Denmark, one can see a clear-cut seasonal trend in prevalence. (Fig. 9.)

In brief, then, there is evidence that

infectious hepatitis has been and still is widespread and endemic throughout Germany and, indeed, Europe and Scandinavia. In Germany hepatitis seems to have flourished, particularly in Bavaria. It has been into the midst of this endemic and epidemic

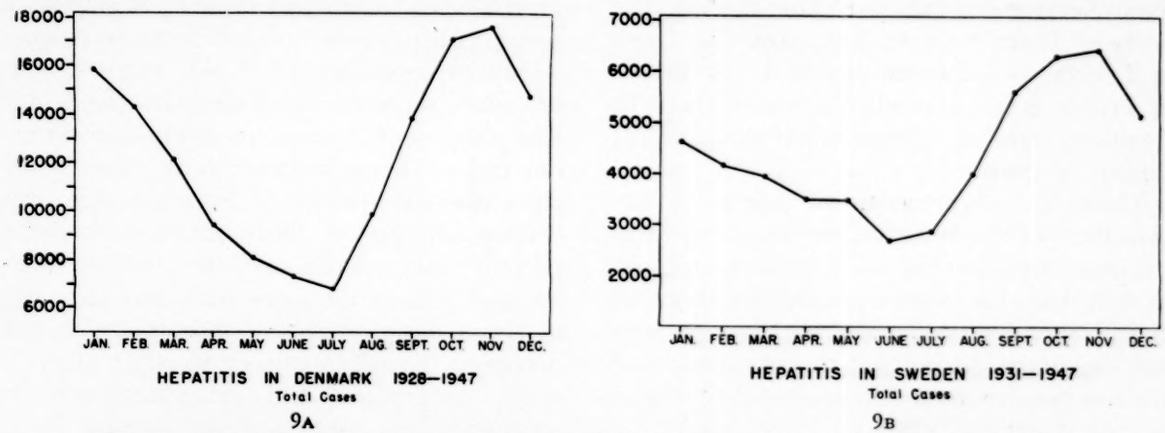


FIG. 9. A, seasonal trends of hepatitis in Denmark (from Havens[36]); B, similar trends in Sweden.

area that U. S. troops have been introduced. It does not seem surprising, therefore, that this disease has spread among them for not only do they occupy Germany but they also occupy the role of susceptible immigrants.

POSTWAR EXPERIENCE WITH HEPATITIS IN U. S. TROOPS IN GERMANY

Locale. During the four and one-half year period 1945–1950, U. S. troops representing the Army of Occupation in Germany have numbered from one hundred thousand to several hundred thousand. They have been stationed in Berlin as well as in the American Zone of Germany, which is an area of some 42,000 square miles comprising a major segment of southwestern Germany and one which is largely occupied by Bavaria. (Fig. 5.) This part of Germany contains a variety of terrain, flat and mountainous, agricultural and industrial, with a number of important cities in the Zone including, in particular, Frankfurt, Heidelberg, Nürnberg and Munich.

The troops have been widely distributed throughout this area during this four and one-half year period and have experienced various types of living conditions either in cities or in small communities or villages. By and large, their contact with German civilians has been increasingly close during the period in which the Occupation has been in force.

Hepatitis Rates. Figure 10 indicates the prevalence of acute hepatitis among U. S. troops in Europe during the past six years (1944–1949), including the combat years in Europe. As mentioned earlier, a high peak was reached (17 per 1,000) in the winter months of the last year of the War in ETO. Subsequently, in the postwar period

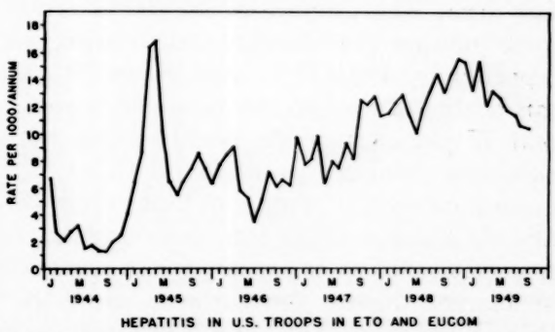


FIG. 10. The rate (per 1,000 per annum) at which hepatitis occurred among U. S. Troops in Europe (ETO) during the years of combat, 1944–1945, and the postwar years of the occupation of Germany (EUCOM) in 1945–1950.

rates fell to about 7 per 1,000 and were maintained irregularly at this level for some two and one-half years. More recently they have begun to rise so that during the winter of 1948–1949 the high rates have even approached those of 1945. The military prevalence of this disease in Germany is considerably above that for U. S. troops in

the United States and in Japan (Fig. 1) as well as in Latin America and the Philippines, and also above the British rates for infectious hepatitis in their Army of the Rhine in Western Germany. The only area where rates for hepatitis in U. S. troops have been consistently higher during the postwar years than in Germany has been in Trieste, but the number of U. S. troops in Trieste is but a small fraction of those in Germany and so a significant comparison cannot be made.

There is, at present, no adequate explanation of the fact that the hepatitis rates are increasing among U. S. troops unless it be that there has been a progressive increase in intimate fraternization in U. S. troops and Germans during the recent years, and this has been more or less universal throughout the American Zone.

As to the *geographic* prevalence within the American Zone, not only have there been few if any local foci but a striking feature is its diffuse distribution and sporadic character. As an exception, there was an outbreak of twenty-six cases in 1947 in two ordnance companies located in the Frankfurt area. The circumstances under which these men became ill were well studied and reported by Potts.[30] It was his belief that this outbreak was primarily water-borne in that 77 per cent of the twenty-six patients admitted drinking water found to be contaminated by the sewage of four communities. As another exception, there was an increased rate of cases among troops stationed in Hanau during the summer of 1948.[27] With these exceptions, one searches in vain for any outbreak in company, troop or regiment which is large enough to be called an epidemic. Cases have been reported from all the areas where American troops are stationed in Germany. The only consistent, appreciable concentration of hepatitis has been in Berlin where there have been no sharply localized outbreaks but the rate in 1948 was about 20 per 1,000 as opposed to 13 per 1,000 in the Zone proper. It should be pointed out that the relatively small number of troops in Berlin are in more prolonged and closer contact with Germans than our troops elsewhere.

Some idea of the scattering of the cases can be gained from the distribution of those hospitalized for hepatitis during the month of October, 1947, when a preliminary review was made by Gauld.[31] A review of seventy-three cases reported between August 15 and September 12, 1947, showed that sixty-five separate units were involved and that they were located in thirty-five different cities. In no instance were there more than two cases reported from a single unit during that period. Only occasionally did a patient give a history of contact with another case in the same unit, and in many of those designated as contacts the attack occurred simultaneously, so that only a small proportion can be considered as secondary or contact cases among their fellow soldiers.

Age of Military Patients with Hepatitis. The relative age distribution of 1,000 patients who were ill with hepatitis in 1947 and 1948 has been reviewed; and when *age specific morbidity rates* are computed, as shown in Figure 11, it is evident that age influences the attack rate, as was noted by Gauld in Italy during 1943–1945.[8] This may reflect the war-time findings that unseasoned troops were the most susceptible. We will not generalize here on this point other than to state that the twenty-year olds have about twice as much hepatitis as do the forty-year olds. One cannot conclude that the twenty-year olds are twice as susceptible as the forty-year olds for exposure may play a large part in this differential incidence.*

Time in Germany Prior to Acquisition of Hepatitis. The average U. S. soldier in Germany is there on a two- to three-year assignment and, consequently, the time at which he develops hepatitis should be

* For these data on age we are indebted to Lt. Col. H. A. Schulze, M.C. of the Preventive Medicine Division, Chief Surgeon's Office, Medical Division, EUCOM. The general age distribution of the U. S. military population has been based on 5 per cent surveys made in December, 1947, and March, June and September, 1948.

viewed in this light. From serial analyses on this point carried out in 1947, 1948 and 1949, the situation has appeared to change, but in 1949 it was evident that most of the hepatitis cases were acquired within six to ten months of the time at which the soldier entered the Occupation Zone. (Fig. 12.)

Seasonal Incidence. Mention has already been made that a sharp increase of hepatitis in the fall of the year is characteristic and was particularly marked during the combat years 1943–1945 in the Mediterranean area[6] (Fig. 6).

Among *crowd diseases* in temperate climates

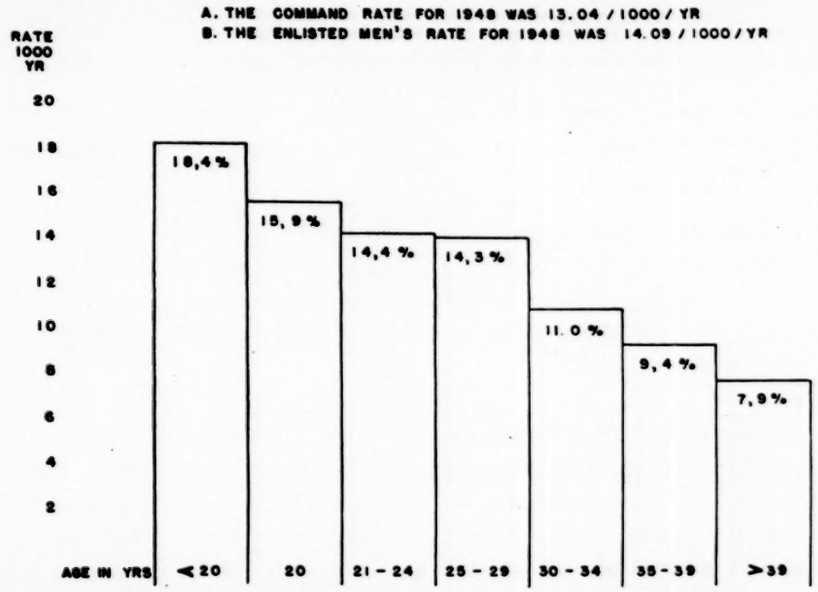


FIG. 11. Age specific hepatitis rates per 1,000 per annum for enlisted men in the American Zone during 1948. Data collected by Lt. Col. H. A. Schulze, M.C.

Racial Incidence. In a series of 1,403 military case records analyzed in 1947–1948 the racial distribution proved to be: 1,224 white patients and 179 Negro. In other words, the Negro cases represented 13 per cent of the total hepatitis cases. This should be compared with the per cent of Negroes within the American Zone which represented in 1947 about 9.5 per cent of the total military population. The differences between 13 per cent and 9.5 per cent are not significant; in other words, one cannot assume that the Negro is either more or less susceptible to hepatitis. If syringe jaundice plays any significant part in the prevalence of hepatitis in troops, one might expect that the Negro rate would be higher than the white because the incidence of venereal disease is higher in Negro troops than in white troops, the rate for syphilis, for instance, being about five times that of whites in the American Zone during 1947.

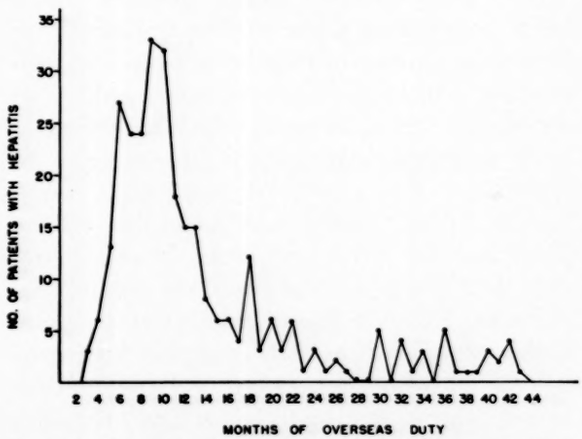


FIG. 12. The time at which U. S. soldiers acquired hepatitis in 1949 during their stay in Germany.

it is customary to expect a seasonal upswing during the wetter and colder months of the year, that is, when people are more apt to be in close contact with one another within doors. The shape and peaks of various seasonal curves differ characteristically for

different diseases and it has long been recognized in the United States that the increase of diphtheria comes in the fall, acute respiratory infections in mid-winter and mumps in the early spring. In this connection it has seemed important to review the seasonal trend of hepatitis in comparison with those of other common diseases* contracted by the military personnel in the American Zone of Germany. For this purpose a monthly compilation representing pooled rates covering the period January, 1946, to June, 1949, has been prepared. (Table III.)

TABLE III

SEASONAL RATES FOR HEPATITIS AND OTHER DISEASES IN U. S. TROOPS IN EUCOM—THE EXPERIENCE OF 1946–1949 IN RATES PER 1,000 PER ANNUM

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Common respiratory	152	180	121	108	92	72	89	83	108	113	116	122
Pneumonia (all types)	9.5	8.7	9.1	5.9	5.7	4.2	5.0	4.4	4.9	5.3	7.7	8.4
Rheumatic fever	1.4	1.5	1.6	1.7	1.8	1.3	1.2	1.4	1.2	1.0	1.2	1.1
Mumps	2.7	3.5	4.2	5.7	4.1	2.0	1.6	1.0	0.7	0.5	0.7	1.1
Dysentery	4.8	3.2	3.6	3.5	5.2	7.4	8.5	8.6	5.2	5.2	5.1	3.7
Hepatitis	9.7	10.9	10.6	8.8	8.0	8.5	8.7	9.5	9.8	11.1	11.2	11.2

In Figure 13 the "winter diseases" have been charted to show their comparison to hepatitis. Common respiratory diseases (including influenza, the common cold and tonsillitis) show, as usual, the highest rates, with a sharp winter rise and a peak in February. As might be expected, pneumonia (of all types) has a similar winter peak but the rates are much lower, being only 5 per cent of the common respiratory diseases. Mumps has the greatest seasonal fluctuation, with a peak in April. Hepatitis has the least fluctuation but had an irregular broad plateau lasting from October through March. Perhaps it could be better described as having two peaks. Nevertheless, the curve here reflects one feature peculiarly characteristic of both military and civilian hepatitis rates, namely, the hepatitis rate arrives at its *highest levels early*, in October or November. It may not start earlier than is the case with common respiratory diseases but it arrives at high levels earlier. Hepatitis, for some unknown reason, in Germany as in other places starts as an autumnal disease.

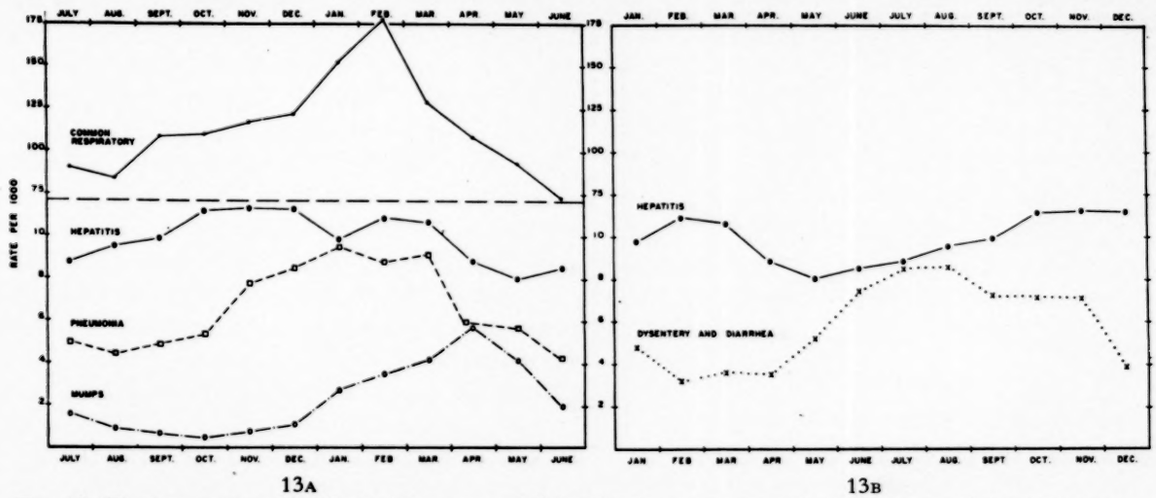
In Figure 13 a summer disease has also been charted, namely, the seasonal trend of dysentery among U. S. troops in the American Zone. The comparison of the dysentery curve to that of hepatitis can be made.

When it comes to the interpretation of the seasonal curve reflecting the monthly prevalence of hepatitis in U. S. troops during the four and one-half postwar years of the Occupation of Germany, we find that the EUCOM* hepatitis curve does not simulate that usually seen among civilian populations. (Fig. 9.) Nor is it the same as that seen during the combat years in Italy, 1943–1945. (Fig. 6.) Nor is it exactly similar to that observed for hepatitis among British Occupation troops during a comparable period. It is true that each autumn the characteristic rise in the hepatitis rate for U. S. troops has occurred, but it has been less precipitous than it was during the combat years in North Africa in 1943–1944, in Italy during 1943–1945 or in Europe in 1944–1945. In the Occupation period in Germany the winters of 1947–1948 and 1948–1949 consistently reveal what might

* It should be mentioned here that during these three and one-half years the rates for diseases other than hepatitis among U. S. troops in Germany, on the whole, have been low.

* The term EUCOM is the designation given to the European Command representing U. S. Troops in Germany.

be called a double peak, the second being a prolongation of the first. (Fig. 10.) This comes out more prominently if the monthly experience of three and one-half years of German Occupation is pooled (Fig. 14) and the U. S. experience is compared with cases, of which a very large percentage had been under treatment in the salvarsan clinic, the peak was "delayed" until January. (Fig. 8.) It would be interesting though speculative to consider what this might mean. Theoretically, there is no particular


JULY AUG. SEPT. OCT. NOV. DEC. JAN. FEB MAR APR MAY JUNE
JAN. FEB MAR APR MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC.
RATE PER 1000
COMMON RESPIRATORY
HEPATITIS
PNEUMONIA
MUMPS
DYSENTERY AND DIARRHEA

13A 13B

FIG. 13. The seasonal trend of hepatitis compared (A) with three common winter diseases and (B) one summer disease, contracted by military personnel in the American Zone during the postwar occupation period.

that of the British from the same period.* U. S. troops show a primary peak in the usual period of September–November and a secondary one during February–March. The British troops show only the autumnal (primary) rise.

This question of seasonal prevalence is a point which we believe to be of some importance. It could reflect the type of disease now prevalent in U. S. troops and conceivably could indicate a change in mode of spread. Such a change would have a tendency either to eliminate or distort the seasonal fluctuations. In our attempts to interpret this bizarre EUCOM seasonal curve, one can recall the previous German experiences with syringe (and/or serum) hepatitis listed in Ruge's studies carried out in the German Navy in the 1920's.[25] In charting the monthly incidence of the various types of "jaundice," he found that in the German Army catarrhal jaundice reached a peak in October; but in his Navy

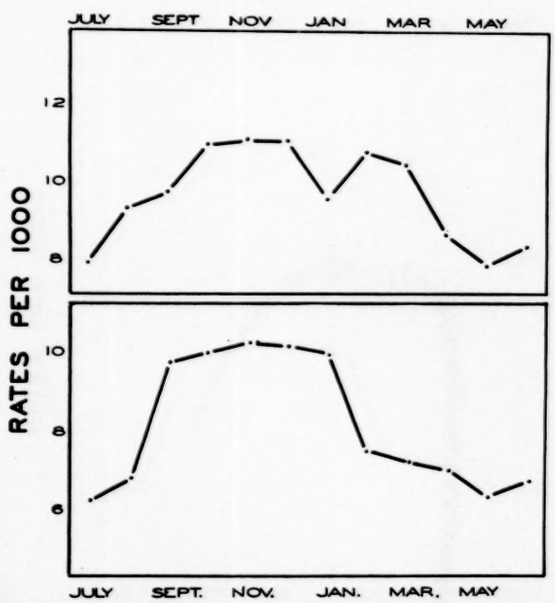


FIG. 14. Seasonal fluctuations in U. S. troops (top panel) and British troops (bottom panel) in Germany. The British curve is more characteristic of infectious hepatitis. A double peak characterizes the U. S. curve.

reason why syringe jaundice should be more prevalent during one month than another unless it be that more injections are given

* We are indebted to Medical Officers of the British Army of the Rhine for these data obtained in 1948 and 1949.

at particular times of the year than at others, or that "viremic donors" are more prevalent at certain particular times. If in the fall of the year more subclinical cases of IH were attending a salvarsan clinic, they would represent an increase of "viremic" donors, and then it would be logical to expect more cases of syringe (and /or serum) hepatitis to occur from one to four months later, and this is what Ruge's January peak suggests. This is speculation but worthy of consideration in connection with the *double peak* noted over several years as occurring among U. S. cases in the American Zone of Germany. (Fig. 14.)

TABLE IV
HISTORY OF INOCULATIONS

	No.	Per cent
Previous transfusions within 6 months prior to onset of hepatitis	38	2.7
Parenteral therapy for syphilis	156	11.1
Parenteral therapy for gonorrhea	246	17.5
Total venereal disease parenterally treated	402	28.6
Total parenteral therapy exclusive of immunizations	440	31.3

Evidence for Syringe Hepatitis in U. S. Cases. Since either serum (SH) hepatitis or infectious (IH) hepatitis can be transmitted parenterally, it was thought that perhaps some clue as to the importance of the syringe in the transmission of the hepatitis in Germany might be obtained by comparing the number of parenteral treatments or inoculations received by patients with hepatitis as compared with those received by the total military (U. S.) population in Germany. A careful inquiry of parenteral injections received during the six-month period prior to hospitalization for hepatitis was therefore made. In the results it became apparent that routine immunizations had been so generally employed that they could not be used for comparative purposes, although they remain, of course, a possible means of transmission. On 1,403 patients studied between September 1, 1947, and July 1, 1948, with inoculation histories covering the period March 1, 1947 to May 1, 1948, the results listed in Table IV were obtained:*

Of these figures, only those pertaining to venereal disease offer something which can be compared to those affecting the general military population of Germany. The rate of parenteral VD treatments in the 1,403 patients may be estimated as 245 per 1,000 per annum. When compared with the VD treatments received during the same period by the total U. S. military population in Germany, this averaged 187 per 1,000 per annum. In other words, 28.6 per cent of hepatitis patients had been treated for venereal disease while 14.2 per cent of the general military population had received such treatment. Conclusions from such a comparison would be subject to grave doubt since we are dealing with a very large, shifting population on the one hand and a relatively small number of patients with hepatitis, but the facts do suggest that parenteral therapy for VD may have increased the number of cases of hepatitis.

Clinical Course. Because of the clinical similarity between infectious hepatitis (IH) and serum hepatitis (SH), there is little information to be expected from analyses of case records with regard to the type or types of disease which have been present in the American Zone. However, attention has been called to the facts that in infectious hepatitis the onset of disease is abrupt and accompanied by fever in a large percentage of patients in contrast to the insidious onset and rarity of fever in serum hepatitis.[32] It is of interest in this regard that of a large group of patients with hepatitis seen at the 120th Station Hospital in Germany in 1947–1948, only 20 per cent had a history of acute onset and, in these, fever was only occasionally recorded.

The relative infrequency of cases with an acute febrile onset might lead to the assumption that the great majority of the cases among the U. S. Occupation troops in

* A similar comparison on 375 of these patients included here has been reported from the Office of the Chief Surgeon, European Command.[33]

Germany are examples of serum hepatitis. Although the clinical findings are not incompatible with this idea, we believe such a conclusion to be unwarranted as yet and that considerably more data and knowledge are necessary before the various types of hepatitis can be differentiated.*

COMMENT AND SUMMARY

Evidence has been presented to indicate that infectious hepatitis has been prevalent and endemic in Germany for many years and, therefore, it is not surprising that this disease should have been common among U. S. troops in the Army of Occupation quartered there during the postwar years. Indeed Bavaria, which occupies the major portion of the American Zone in Germany, has in the past been a favored area for hepatitis in comparison to Prussia, for instance.[25] With the entry and maintenance of U. S. troops in this region, we have an example of a continuous supply of susceptible immigrants constantly mingling with an "infected population." It is our suspicion that many if not the majority of U. S. soldiers who acquire hepatitis in Germany do so as a result of direct personal contact with German people or in German households. But coupled with the natural disease, it would also seem highly probable that the infection is maintained in the military populations by the fact that many cases of infectious hepatitis (IH) as well as serum hepatitis (SH) are transmitted artificially via needles and syringes. The evidence for this is circumstantial and the extent to which this may be so is an unknown quantity, but there is ground for suspicion. For instance, the seasonal curve of hepatitis among U. S. troops has more or less consistently shown some deviations from the usual autumnal rise and early winter decline of infectious hepatitis cases as seen in civilian populations and among troops in combat observed previously and elsewhere. These deviations are compatible with the idea that some of the winter cases may have been artificially transmitted. However, until there is further proof that the artificial transmission of hepatitis is actually of major significance, it may not be wise to discuss it further, although it represents a working hypothesis on which to base future epidemiologic investigations as well as an hypothesis on which to base a program aimed at the prevention or the lessening of the high incidence of this disease among our Army of Occupation in Germany.

* Experimental proof of the nature of the hepatitis among these troops has been obtained to date on materials from only two military patients in Germany. The incubation periods of volunteers who contracted hepatitis after experimental inoculation fell within the range of infectious hepatitis (IH) in the series of tests on material from one of these patients,[34] and within the range of serum hepatitis (SH) in another.[35] There is definite evidence, therefore, that serum hepatitis exists among U. S. troops in EUCOM.

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An Evaluation of Two Screening Tests for the Detection of Early and Sub-icteric Viral Hepatitis*

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THE lack of a specific immunologic test for viral hepatitis makes it necessary to rely on clinical and epidemiologic evidence in addition to certain tests of hepatic function for diagnosis. When jaundice occurs the problem is usually not difficult, particularly in younger patients. However, when jaundice is absent the diagnosis of viral hepatitis is frequently not suspected. It is not known how often non-icteric hepatitis occurs[1] and estimates of its frequency have ranged from one to eight persons for every patient with jaundice.[2] Actually, it is entirely possible that mild forms of disease without icterus may occur far more frequently than is expressed by such ratios. There is good evidence to suggest that at least one form of the disease, infectious hepatitis, is widely distributed. The fact that normal human gamma globulin, administered parenterally, will prevent this disease when given during the incubation preiod suggests that the virus is diffusely spread and that infection in man, with resultant production of immune substances, occurs frequently.[3,4]

Although no specific serologic test is available, certain tests of hepatic function become positive early in the pre-icteric phase of disease. Thus it has been shown that bilirubin and increased amounts of urobilinogen often appear in the urine as the first demonstrable evidences of viral hepatitis.[5] Since the tests for these two substances are easy to perform, an attempt was made to evaluate their usefulness in detecting early or non-icteric cases of viral hepatitis in persons suspected of having the disease or in groups in which cases with jaundice had occurred. It is the purpose of this paper to report the results of such tests in three groups of American troops in Germany. This study was undertaken by the Hepatitis Research Team in co-operation with the 4th General Medical Laboratory and the Branch of Preventive Medicine, Office of the Chief Surgeon, European Command, U. S. Army.

METHODS AND MATERIALS

Subjects. Three groups of men were selected for study. Group I was comprised of men in two Engineer Companies in Hanau, Germany, who had been having a greater than expected incidence of viral hepatitis for several months. Specimens of urine from 388 men present for duty were tested for bilirubin and urobilinogen at the beginning of the study, specimens from 399 men two weeks later and from 380 men six weeks later. Group II was comprised of twenty-two men in an Air Force Squadron in Hof, Germany, who had sustained a small outbreak of food-poisoning. They were tested three weeks after the outbreak because one man developed viral hepatitis at this time. One month later, specimens of urine from these men were tested again. Group III was comprised of fifteen men

* From the Section of Preventive Medicine, Yale University School of Medicine, and the 120th Station Hospital (Hepatitis Research Team), European Command, U. S. Army of Occupation in Germany. Representing work done under the auspices of the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, Office of The Surgeon General, U. S. Army, Washington, D. C.

in an Infantry Regiment in Bamberg, Germany, who reported to their dispensary with complaints suspicious of early viral hepatitis. These men were admitted to the hospital for study and treatment.

TABLE I
RELATIONSHIP OF BILIRUBINURIA, INCREASED UROBILINOGENURIA AND THE DEVELOPMENT OF VIRAL HEPATITIS IN MEN EXPOSED TO THE DISEASE

Group	Time of Test	No. of Men			
		Tested	With Bilirubinuria	With Increased Urobilinogenuria	Developed Viral Hepatitis
I	Initial	388	1	10	1
	2 wk. later	399	1	8	1
	4 wk. later	380	0	9	0
II	Initial	22	0	4*	0
	4 wk. later	22	0	5*	0
III	Initial	15	8	15	8

* Equivocally positive.

In Groups I and II specimens of urine were collected early in the morning and in Group III specimens were collected in the early afternoon. A qualitative test for urobilinogen was done on each specimen from subjects in Group I by adding 2.0 ml. Ehrlich's reagent to 2.0 ml. urine in a Wassermann type test tube. This was mixed, allowed to stand for one minute, and 4.0 ml. of saturated sodium acetate solution were added. The presence of more than a faint pink color to the eye was graded as positive from 1 to 4 plus. The same method was used for the tests on the subjects in Group II except that the reading was done in a Coleman Junior Photometer, according to the method of Schwartz et al.[6] The tests for urobilinogenuria performed on patients in Group III were read in a comparator block.* The presence of bilirubin in the urine was determined according to a modification of Harrison's test.[7]

Each person whose urine contained bilirubin or increased amounts of urobilinogen had the following tests of hepatic function performed during the ensuing week: the one-minute and total serum bilirubin,[8] cephalin-cholesterol flocculation,[9] thymol turbidity,[10] and bromsulfalein retention tests.[11]

RESULTS

The relationship of the results of the tests for bilirubinuria and urobilinogenuria to the development of viral hepatitis in the three groups of men is recorded in Table I.

Group I. Specimens from twenty-seven men had increased amounts of urobilinogen and two of them contained bilirubin. The other tests of hepatic function which were made in these twenty-seven men from one to six days later were normal in twenty-one men. Six men had an increase above normal in one or more tests. Two of these men who were asymptomatic at the time when they had bilirubinuria and increased urobilinogenuria developed viral hepatitis. One of them had jaundice; the other man remained asymptomatic but still had increased retention of bromsulfalein one month later. A third man had increased retention of bromsulfalein (9.25 per cent) but unfortunately a second determination was not made so that no definite conclusion can be made about him. Three others had transitory, slight increases in serum bilirubin (two) and retention of bromsulfalein (one) about which it is impossible to draw conclusions although it is not likely that the alterations were of any significance.

Group II. None of the twenty-two subjects in this group had positive tests for bilirubin at the first examination; four of them had equivocal increases in urobilinogenuria. When these tests were repeated in the same persons one month later, the tests for bilirubinuria were again negative. Of the four who previously had equivocal increases in urobilinogenuria, three were normal and one remained equivocal. Four others previously normal had equivocally positive tests for urobilinogenuria on the second examination. Other tests of hepatic function in these five persons who showed equivocally positive tests for urobilinogenuria at the time of the second examination were all normal except for one obese subject whose retention of bromsulfalein was 6.4 per cent or greater on three occasions.* None of the persons in Group II developed clinical evidence of hepatitis during the

* Will Corp., Rochester, N. Y., No. 17185, Urobilinogen Comparator Set.

* Five per cent retention (45 minutes after the intravenous injection of 5 mg. dye/kg. body weight) is regarded as the upper limit of normal as the test is performed in this laboratory.

month between tests nor for several months following the period of observation.

Group III. Eight of the fifteen men in this group developed viral hepatitis. Six of them had bilirubinuria and all had increased urobilinogenuria. Of the remaining seven men who failed to develop any additional evidence of hepatitis, bilirubinuria was present in two, and all had an increased amount of urobilinogen in the urine. It is possible that some or all of these persons may have had transitory non-icteric hepatitis.

COMMENT

The data presented here indicate that the demonstration of bilirubinuria and increased urobilinogenuria in ten patients exposed to viral hepatitis was of value in the early diagnosis of hepatitis which subsequently developed with jaundice in nine and as the non-icteric disease in the tenth person. It is possible that certain other persons with transitory, mild alterations in various tests of hepatic function represented cases of non-icteric viral hepatitis. The test for bilirubinuria is applicable for use in large numbers of persons due to its ease of performance. In addition, in the studies reported here bilirubinuria was a far more reliable sign of hepatitis than increase in urobilinogenuria although in three patients the latter test was positive before bilirubinuria occurred. This is in accord with the results of others and suggests that both tests be employed for detection of early or non-icteric cases. Since there is no specific serologic test available, the use of these tests, particularly in localities where viral hepatitis occurs in epidemics or is of frequent sporadic occurrence, may yield information of epidemiologic value.

SUMMARY

1. Tests for bilirubin and urobilinogen were performed on 1,226 specimens of urine from American soldiers who had been exposed to viral hepatitis.

2. These tests were of value in making an early diagnosis in the pre-icteric phase of nine patients who subsequently became jaundiced and in one patient with non-icteric hepatitis. Eight of these patients had symptoms suggestive of early viral hepatitis when the tests were made.

3. The apparent reliability and ease of performance of these tests suggest their possible value in epidemiologic surveys of groups exposed to viral hepatitis.

Acknowledgment is made herewith of the assistance from the following individuals: Lt. Col. Hartwin A. Schulze, Preventive Medicine Branch, Chief Surgeon's Office, EUCOM, and Capt. Emil W. Johnson, T/Sgt. Clyde F. O'Brien, S/Sgt. Arnold A. Waldschmidt and Dr. H. Seeliger, all from the 4th General Medical Laboratory, EUCOM.

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Serum Cholesterol and Cholesterol Esters in Viral Hepatitis*

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STUDIES of the partition of cholesterol in the serums of patients with infectious hepatitis have been recorded by several workers[1–4] but most reports have included infectious hepatitis along with other diffuse parenchymatous hepatic diseases, often without clear delineation. More recently Man[5] and her collaborators described the alterations in the serum lipids in a number of hepatic diseases, among them eleven cases of infectious hepatitis, confirming earlier observations on the relative decrease in the proportion of esterified cholesterol early in this disease. They also suggested the possibility that the difference between the hyperlipemic and hypolipemic patients observed in this group might depend on etiologic factors.

Increase in the amount of alkaline phosphatase occurs frequently in the serums of jaundiced patients. Although this alteration is not nearly as great or as constant in viral hepatitis as it is in patients with jaundice due to extrahepatic biliary obstruction, it has been described in cholangiolitic hepatitis and cirrhosis and in chronic hepatitis.[6] The exact cause or causes of increased serum alkaline phosphatase in hepatic disease have not been defined and the physiology of the enzyme has not been clarified despite numerous experimental and clinical investigations.[6–12] Because of the concomitant increases in amounts of cholesterol and alkaline phosphatase which frequently occur in the serums of patients with obstructive jaundice, it was thought to be of interest to compare the type and degree of alteration of these substances in patients with viral hepatitis. It is the object of this article to describe such observations made on a large group of patients with this disease.

MATERIAL AND METHODS

Subjects. Six hundred twenty-three soldiers who had contracted viral hepatitis while on duty during 1947 to 1948 with the U. S. Army of Occupation in Germany were studied. Their average age was 22.5 years; all were well nourished and presumably healthy before the onset of disease. The average length of hospitalization was between five and six weeks and patients remaining longer than this usually had a more severe course of disease with a slower return to health. Attention is called to this fact, particularly in relation to the observations made in the later weeks of disease.

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Protocols. Brief histories of four patients with viral hepatitis are recorded, together with the results of certain tests of hepatic function. (Fig. 1.) Two of these patients (C. H. and W. D.) had had no parenteral therapy or dental work during the eight-

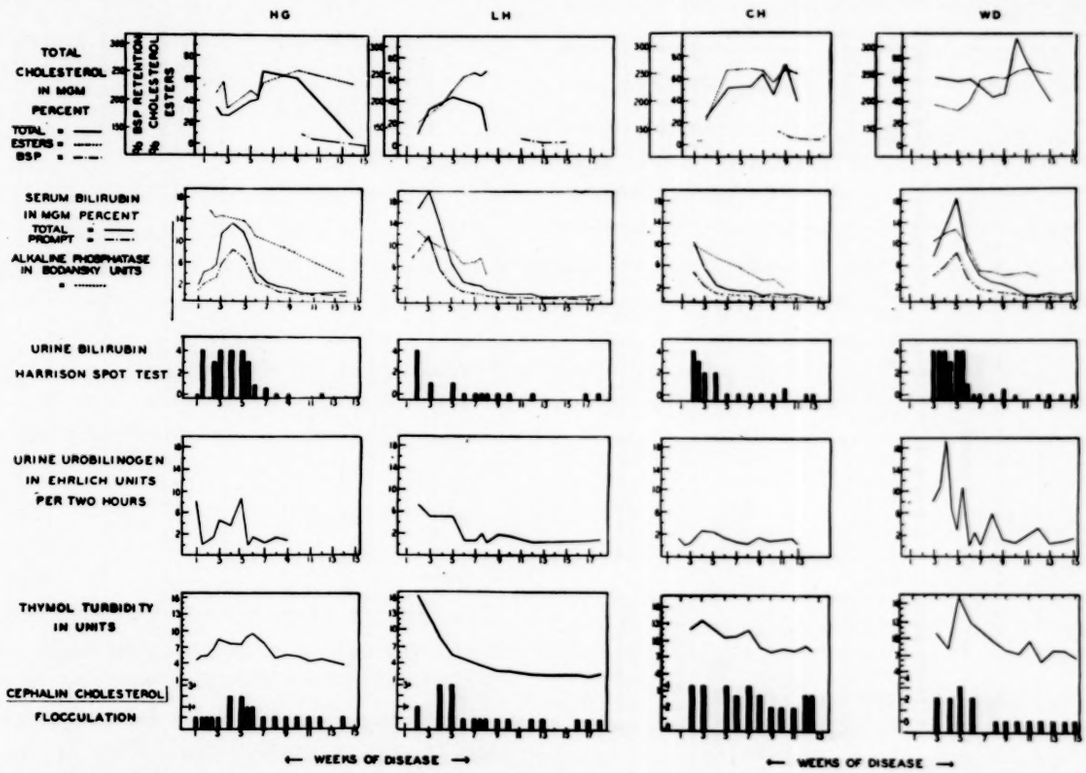

FIG. 1. Results of partition of serum cholesterol and determinations of serum alkaline phosphatase and other tests of hepatic function in four patients with viral hepatitis.

month period prior to admission and presumably represent the naturally occurring disease. Two others (H. G. and L. H.) had received parenteral therapy with penicillin two and four months before onset of hepatitis so that the diagnosis of serum hepatitis cannot be excluded.

CASE I. C. H., a forty year old white male who had consumed moderate amounts of alcohol for several years, became sick on January 21, 1948, with diarrhea and dark urine. Nausea, vomiting, chilly sensations and fever appeared the following day. These symptoms persisted until the twenty-sixth when jaundice was first noted. On admission to the hospital on the thirtieth, jaundice was the only abnormal physical finding. The stools were acholic for several days, becoming normal on February 6th. He was discharged from the hospital asymptomatic on March 31st although positive flocculation tests and abnormal retention (7.25 per cent in forty-five minutes) of bromsulfalein were present. Subsequent studies on April 12th and May 3rd revealed evidence of possible residual hepatic damage as manifested by persistently positive flocculation tests and abnormal retention of bromsulfalein. He was asymptomatic at this time.

CASE II. W. D., a twenty year old white male, had anorexia and dark urine about January 1st, 1948, and on the twelfth icterus appeared. Physical examination on admission to the hospital, January 16th, revealed deep jaundice and a barely palpable, non-tender liver. One "spider" angioma was observed on his neck. Acholic stools were present until February 4th and mild pruritus occurred. His appetite returned about February 7th and he apparently made an uneventful recovery and was discharged April 9th, 1948.

Case III. H. G., a thirty-five year old white male, was given parenteral treatment with penicillin for pneumonia on November 8, 1947.

He recovered rapidly and was well until January 21, 1948, at which time he noted malaise, irritability, diarrhea and vomiting. Bilirubinuria became evident on January 23rd, and scleral icterus was observed on the twenty-fifth. Physical examination on admission to the hospital revealed mild anterior cervical, axillary and inguinal adenopathy and moderate tenderness of the liver which was palpable 8 cm. below the right costal margin. Jaundice increased and his course of disease was severe although recovery was uneventful.

TABLE I
SERIAL PARTITIONS OF CHOLESTEROL IN THE SERUMS OF NINE HEALTHY SOLDIERS

Subject	Days	Total Cholesterol (mg./%)	Cholesterol Partitions	
			Esterified (mg./%)	Percentage of Esters
SH	1	223	161	72
	2	210	147	70
	3	222	154	68
	4	222	150	67
	5	236	157	66
HE	1	137	101	73
	2	145	117	76
	3	140	95	68
	4	150	100	67
	5	131	88	67
AL	1	137	94	69
	2	146	145	82
	3	156	111	71
	4	144	94	65
	5	200	135	68
FH	1	193	136	70
	2	171	118	69
	3	239	159	64
	4	156	99	63
	5	177	113	64
DR	1	181	125	69
	2	178	116	65
CA	1	104	69	66
	2	116	69	60
PU	1	151	106	70
	2	149	97	65
BO	1	133	80	60
	2	190	131	69
LA	1	250	177	71
	2	271	197	73

CASE IV. L. H., a twenty year old white male, was given parenteral therapy with penicillin for syphilis in August, 1947. He was well until January 25, 1948, when he observed dark urine. The following day nausea and jaundice appeared. Physical examination on admission to the hospital, February 6th, revealed deep jaundice. The liver was tender and palpable 4 cm. below the right costal margin. His course of disease was uneventful and he was discharged asymptomatic on May 14th.

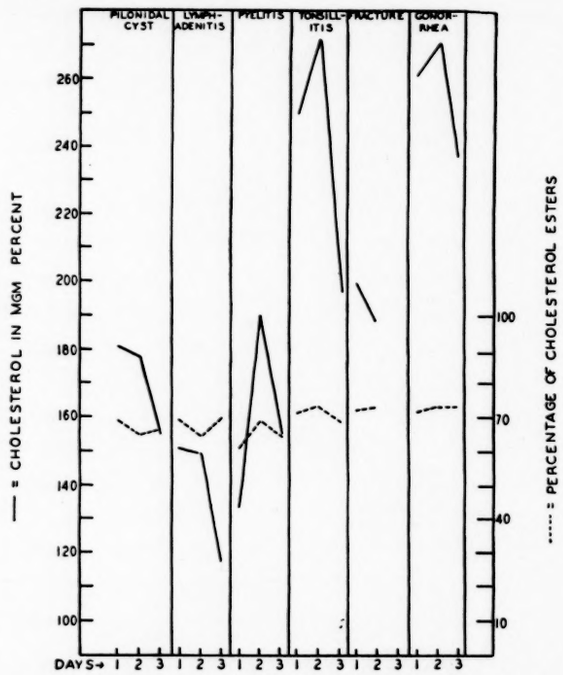

FIG. 2. Partition of cholesterol in the serums of six patients with diseases other than hepatitis.

Laboratory Observations. In order to obtain a representative sampling at regular intervals during the course of hepatitis, 100 random determinations of the amounts of cholesterol and esters and alkaline phosphatase were made on unselected serums obtained from patients in each week of the disease. One hundred seventy-two patients had three or more serial determinations made at weekly intervals and the remainder had single random determinations. It was not possible to obtain 100 serums during the period of this study from patients in the first week of disease because of the frequent delay in transfer of patients from other hospitals to the Hepatitis Center.

Control determinations of cholesterol were done on serums obtained on several successive days from nine healthy soldiers (Table I) and from six patients with diseases other than hepatitis. (Fig. 2.) Single determinations were also made on sixty-eight

patients hospitalized for other diseases on the medical and surgical wards. (Tables II and III.) Hourly determinations were made on five normal soldiers on the same day for four consecutive hours. (Fig. 3.)

The control determinations on normal persons, although showing some day-to-day fluctuation, are almost all within the rather narrow range which has been found consistently by Peters and Man[13] and Sperry[14] in normal healthy persons. A larger series of control determinations on samples of serum from patients with other diseases on the medical and surgical wards includes some results, however, in which the ratio of free: total cholesterol was greater or less than this constant normal range (0.24 to 0.32). Such variation in hospitalized patients with other than hepatic disease has not been previously described[15] and one can only speculate as to its cause. It has been suggested that it might be related to surreptitious consumption of alcohol.[15] Whatever the cause of these few variations the general pattern of response in the control subjects illustrates the constancy of the ratio of free: total cholesterol which has been emphasized by others[13,14] despite the considerable variation from hour to hour in the amount of total cholesterol.

TABLE II

PARTITIONS OF CHOLESTEROL IN THE SERUMS OF THIRTEEN PATIENTS HOSPITALIZED ON SURGICAL WARDS FOR DISEASES OTHER THAN HEPATITIS

Subject	Total Cholesterol (mg./%)	Cholesterol Esters (mg./%)	Esters (%)	Ratio Free:Total
1	270	197	73	.27
2	200	140	70	.30
3	150	103	69	.31
4	183	130	71	.29
5	133	93	70	.30
6	150	103	69	.31
7	133	96	72	.28
8	200	153	77	.23
9	233	173	74	.26
10	150	104	69	.31
11	133	96	72	.28
12	200	147	74	.26
13	150	103	69	.31

TABLE III

PARTITIONS OF CHOLESTEROL IN THE SERUMS OF FIFTY-FIVE PATIENTS HOSPITALIZED ON THE MEDICAL WARDS WITH DISEASES OTHER THAN HEPATITIS

Subject	Total Cholesterol (mg./%)	Cholesterol Esters (mg./%)	Esters (%)	Ratio Free:Total
1	200	147	74	.26
2	150	99	66	.34
3	167	120	72	.28
4	250	165	66	.34
5	150	110	73	.27
6	233	173	74	.26
7	215	148	68	.32
8	150	57	62	.38
9	150	97	65	.35
10	167	111	66	.34
11	167	120	72	.28
12	237	172	73	.27
13	127	87	63	.37
14	200	147	73	.27
15	133	93	71	.29
16	127	81	63	.37
17	150	97	65	.35
18	200	125	63	.37
19	250	163	65	.35
20	183	136	74	.26
21	167	120	72	.28
22	167	114	68	.32
23	150	103	69	.31
24	183	143	78	.22
25	200	127	64	.36
26	200	147	74	.26
27	250	183	73	.27
28	200	140	70	.30
29	133	80	60	.40
30	130	88	67	.33
31	167	114	68	.32
32	200	140	70	.30
33	183	143	78	.22
34	129	83	65	.35
35	167	114	68	.32
36	137	90	66	.34
37	150	113	75	.25
38	183	123	67	.33
39	176	114	65	.35
40	183	123	67	.33
41	200	140	70	.30
42	178	116	65	.35
43	256	186	73	.27
44	133	93	71	.29
45	200	147	75	.25
46	167	127	76	.24
47	127	92	72	.28
48	233	120	69	.31
49	187	127	68	.32
50	117	84	72	.28
51	157	110	70	.30
52	133	93	70	.30
53	183	143	78	.28
54	138	91	65	.35
55	150	57	62	.38

Total cholesterol range (256 − 117 mg. %) = 139 mg. %
Cholesterol esters range (186 − 57 mg. %) = 129 mg. %
% Cholesterol esters range (78 − 60%) = 18%
Mean 69.2
Median 70.0
Average deviation from median .. 3.78
Standard deviation from mean ... 4.423

The amount of alkaline phosphatase in the serum was determined according to the method of Bodansky.[16] The method of Schoenheimer and Sperry[17] was used for the determinations of cholesterol, digitonin being used in the precipitation. The blood

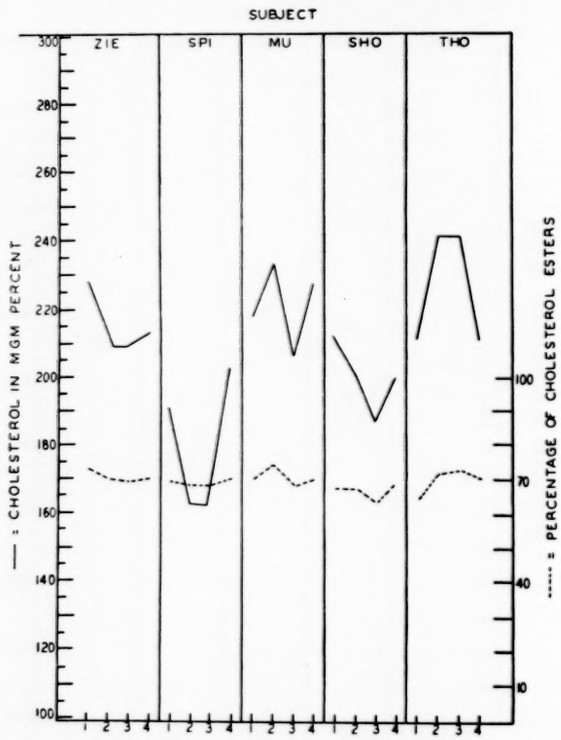

FIG. 3. Hourly determinations of cholesterol in the serums of five normal persons, illustrating the constancy of the ratio of free:total cholesterol.

was drawn in the usual manner thirty to sixty minutes after breakfast. The interval of time between collection of samples and precipitation averaged between two and three hours. Since it has been shown[14,18] that there is a decrease in the amount of free cholesterol in blood after prolonged standing or incubation, it would appear that this delay would cause, if anything, an increase of the cholesterol esters at the expense of free cholesterol. Any "Estersturz" found should, therefore, represent a true decrease.

RESULTS

The alterations in the partitions of cholesterol and a comparison of them with the alterations in serum alkaline phosphatase in 623 patients with viral hepatitis are recorded. (Figs. 4 and 5.) It is apparent that despite the relative paucity of determinations in the first week of hepatitis, there is a decrease of the percentage of cholesterol esters in the early weeks of the disease, with a gradual return toward normal levels during convalescence. However, it is also evident that in the later weeks of the disease the ratio of free:total cholesterol is often still elevated. The fact that a number of these ratios are outside the normal limits (0.24 to 0.32) in the later weeks of the disease is probably best explained by the point that patients remaining in the hospital (and thus available for study) later than the fifth or sixth week of disease have had an unusually severe course of disease or a more delayed recovery. None of the patients in this series showed a true hypercholesterolemia and only three patients had a hypocholesterolemia with total cholesterols below 100 mg. per cent during the first two weeks of the disease.

The amount of alkaline phosphatase in the serum is somewhat greater than is usually reported in the acute phase of disease, returning to essentially normal levels in convalescence. The curve of average determinations of serum alkaline phosphatase shows a decrease after the early period of disease, roughly reciprocal to the curve of averaged determinations of cholesterol esters. (Fig. 5.)

SUMMARY

1. Determinations of the partition and amounts of cholesterol and alkaline phosphatase in the serums of 623 patients in the first ten weeks of viral hepatitis were made.

2. The serum cholesterol esters were diminished and the serum alkaline phosphatase increased somewhat during the early weeks of the disease. Both tended to become normal during convalescence.

3. True hypercholesterolemia was not recorded and hypocholesterolemia was rare in these patients.

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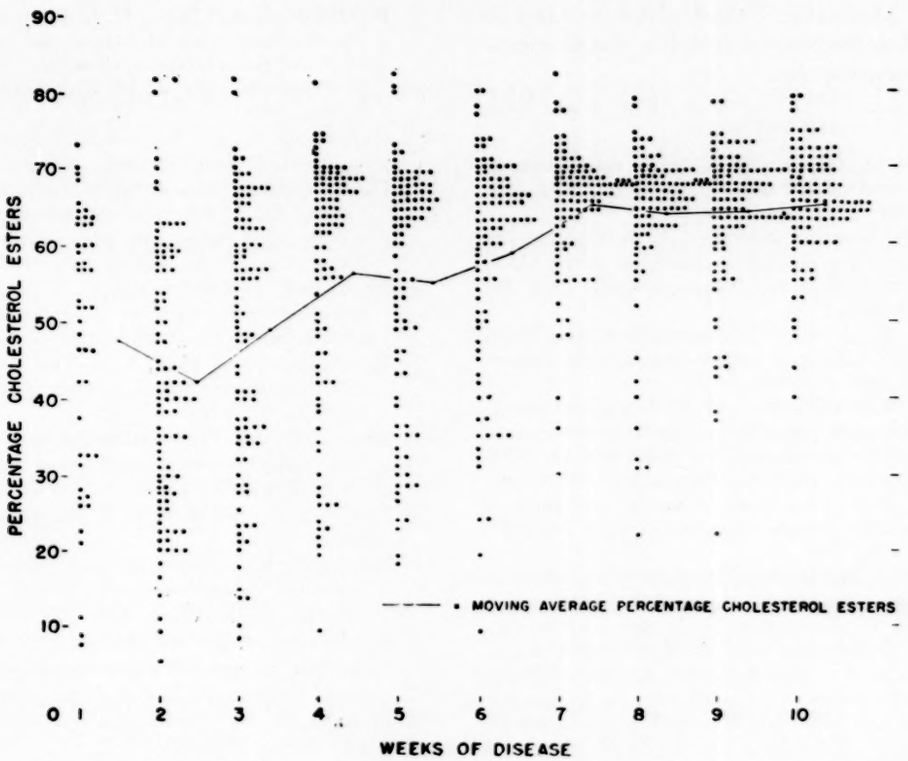

FIG. 4. Percentage of cholesterol esters in patients with hepatitis during the first ten weeks of the disease.

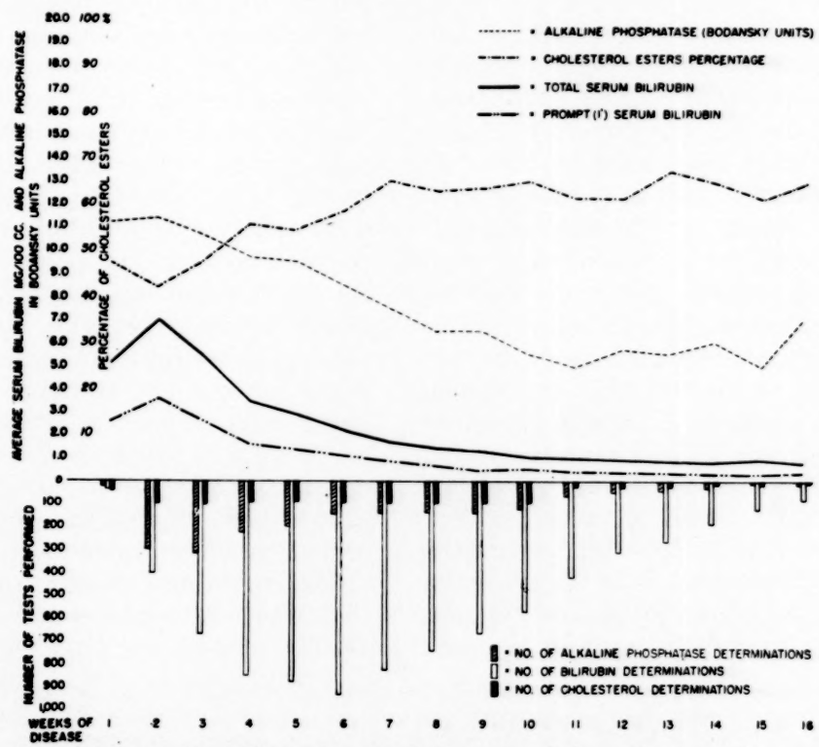
AVERAGE SERUM BILIRUBIN MG/100 CC. AND ALKALINE PHOSPHATASE IN BODANSKY UNITS
PERCENTAGE OF CHOLESTEROL ESTERS
ALKALINE PHOSPHATASE (BODANSKY UNITS)
CHOLESTEROL ESTERS PERCENTAGE
TOTAL SERUM BILIRUBIN
PROMPT (1') SERUM BILIRUBIN
NUMBER OF TESTS PERFORMED
NO. OF ALKALINE PHOSPHATASE DETERMINATIONS
NO. OF BILIRUBIN DETERMINATIONS
NO. OF CHOLESTEROL DETERMINATIONS
WEEKS OF DISEASE

FIG. 5. Averaged partitions of serum cholesterol and determinations of serum alkaline phosphatase and 1′ direct and total serum bilirubin.

Professor of Medicine, Yale University, and to Dr. E. B. Man for their counsel in the preparation of this manuscript.

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Factors Influencing Retention of Bromsulfalein in the Blood of Patients with Viral Hepatitis*

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The removal of intravenously injected bromsulfalein from the blood is generally believed to be effected by two mechanisms: (1) the reception of the dye in a storage space and (2) the transfer of the dye into the biliary system. Although it has been concluded by several workers[1–4] that the reticuloendothelial system is concerned with the removal of the dye from the blood, there is no clear evidence that the extrahepatic reticuloendothelial cells are involved significantly,[5,6] and it has been suggested that the Kupffer cells in the liver provide the main storage depot for dye after intravenous injection.[6] Many of the factors which influence the removal of bromsulfalein from the blood are poorly defined; although it is thought that in patients with hepatic disease, decreased removal of dye may be ascribed to (1) an impaired cellular mechanism for removal or (2) a decreased flow of blood to functioning hepatic tissue, or a combination of both.[6]

The use of this test of hepatic function in large numbers of patients with viral hepatitis provided data which are of interest when correlated with certain conditions which might influence the results of the test. It is the purpose of this paper to describe (1) the effect of ingestion of food on the removal of bromsulfalein from the blood of patients with viral hepatitis and (2) the relationship between the percentage of retention of dye in the blood and various amounts of 1′ direct and total serum bilirubin in such patients.

Effect of Ingestion of Food on Removal of Bromsulfalein from the Blood

The effects of alterations of the flow of hepatic blood have been neglected in evaluating hepatic function, and actually it is only recently that an adequate method for estimation of hepatic blood flow has been available in man.[7] It has been shown that the capacity to remove bromsulfalein from the blood diminishes in normal persons when the position is shifted from supine to upright. In contrast, the administration of adrenalin increases the removal of dye, possibly due to an increased flow of hepatic blood.[6] The effect of ingestion of food on the removal of dye from the blood has not been widely studied, but it has been thought to have little influence.[6] Recent studies in this laboratory revealed that ingestion of food apparently altered the rate of removal of bromsulfalein from the blood of patients with viral hepatitis and

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also of normal persons. The results of these studies are reported below.

Materials and Methods

Subjects. The patients studied in these investigations were in an Army hospital in Germany at some time during the period from September, 1947, to March, 1949. They had contracted viral hepatitis during the performance of duty. It is quite probable that many of these cases were examples of homologous serum hepatitis; but in view of the fact that there are no means at present for differentiating infectious hepatitis from serum hepatitis clinically, the cases have all been classified as viral hepatitis. The diagnosis was made on the basis of characteristic symptoms and signs, accompanied by consistent deflection of appropriate tests of hepatic function.

Three groups of persons were utilized for the study of the effect of ingested food on the removal of bromsulfalein from the blood. Group I was comprised of forty-eight young male patients convalescing from viral hepatitis, in a period of disease ranging from the 17th to the 100th day. The 1′ direct serum bilirubin ranged from 0.07 mg. per cent to 0.93 mg. per cent, and the retention of bromsulfalein ranged from 1.44 per cent to 20 per cent at the beginning of the study. Group II was comprised of forty-four young male patients convalescing from viral hepatitis, in a period of disease ranging from the 19th to the 39th day. The 1′ direct serum bilirubin ranged from 0.24 mg. per cent to 0.80 mg. per cent, and the retention of bromsulfalein ranged from 1.44 to 18 per cent at the beginning of the study. Group III was comprised of twenty-six apparently healthy young adult males.

Laboratory Observations. In Group I three determinations of the percentage of retention of bromsulfalein in the blood were made in each patient, with intervals of seven days between each test. The first and third tests were performed sixty to ninety minutes after the ingestion of a breakfast containing 215 gm. carbohydrate, 28 gm. protein, and 34 gm. fat (1,278 calories). The second or intermediate test was performed with the patients fasting.

In Group II three determinations of the percentage of retention of bromsulfalein in the blood were made in each patient, with intervals of seven days between each test. The first and third tests were performed sixty to ninety minutes after the ingestion of the same breakfast given in Group I. The second or intermediate test was performed immediately following the intravenous administration of 0.5 gm. of glucose/kg. of body weight, given in 10 per cent solution in sterile distilled water at a constant rate over a period of thirty minutes.

TABLE I

MEAN AMOUNTS OF 1′ DIRECT SERUM BILIRUBIN IN TWO GROUPS OF PATIENTS WITH VIRAL HEPATITIS TESTED AT WEEKLY INTERVALS

Group	1′ Direct Bilirubin—mg./100 ml. Serum		
	Test 1	Test 2	Test 3
I (48)*	0.396	0.290	0.222
II (44)*	0.436	0.323	0.240

* Number of patients in each group.

In Group III two determinations of the percentage of retention of bromsulfalein in the blood were done in each person, with an interval of two days between the tests. The first test was done sixty to ninety minutes after the same breakfast given in Groups I and II. The second test was done with the patients fasting.

Determinations of the 1′ direct and the total serum bilirubin were made in each patient in all three groups in the fasting state on every day on which the degree of retention of bromsulfalein was measured. The determinations of the serum bilirubin (1′ direct and total) were done according to the method of Malloy and Evelyn[8] modified by Ducci and Watson[9] with the exception that each of the two types of bilirubin was determined in a separate tube. The percentage of retention of bromsulfalein in the blood was measured forty-five minutes after the introduction of dye in amount of 5 mg./kg. of weight of the patient.[10] All determinations were made in a Coleman spectrophotometer.

Results

During the three successive weekly tests on patients in Groups I and II the mean amounts of 1′ direct serum bilirubin declined progressively toward normal. (Table I.) Of the ninety-two patients in these two groups, only eight failed to have a lessened amount of 1′ direct serum bilirubin between the first and the second test, and

only three failed to decline between the first and the third test. Each of these three patients either remained the same or had a maximum rise of 0.07 mg. per cent, and all three determinations were normal or slightly above normal. Similarly, the mean

TABLE II

MEAN PERCENTAGE OF RETENTION OF DYE IN THE BLOOD FORTY-FIVE MINUTES AFTER THE INTRAVENOUS ADMINISTRATION OF BROMSULFALEIN IN TWO GROUPS OF PATIENTS WITH VIRAL HEPATITIS TESTED AT WEEKLY INTERVALS

Group	Percentage of Retention of Bromsulfalein		
	Test 1	Test 2	Test 3
I (48)*	8.84	11.25†	6.28
II (44)*	8.95	11.53‡	5.51

* Number of patients in each group.
† Test performed in fasting state.
‡ Test performed after intravenously administered glucose.

percentage of retention of bromsulfalein in the forty-eight patients in Group I and of the forty-four patients in Group II was lower at the time of the third test than at the first. (Table II.) The decline in amount of serum bilirubin and retention of bromsulfalein was accompanied by clinical improvement, indicating the progressive convalescence of these patients.

It is of interest then to observe that the results of the second or intermediate determination of retention of bromsulfalein showed an increase in the mean percentage of retention of dye, both in Group I in which the test was done in the fasting state, and in Group II in which the test was performed following the intravenous injection of glucose. (Table II.) The mean increase in percentage of retention of dye in the second tests was the same in both groups of patients and was statistically significant, even when the mean values of the second tests were compared with those of the first tests rather than with a calculated value intermediate between the first and third tests.

In Group III consisting of twenty-six normal subjects the mean percentage of retention of bromsulfalein in forty-five minutes was 2.81 per cent when measured postcibum and 3.66 per cent in the fasting state. Only one subject had retention of dye greater than 5.0 per cent (5.25 per cent) when tested postcibum, whereas five tested in the fasting state had retention of more than 5 per cent (5.5, 5.9, 6.5, 7.0 and 7.1 per cent).

RELATIONSHIP OF BILIRUBINEMIA AND RETENTION OF BROMSULFALEIN IN THE BLOOD

The effect of hyperbilirubinemia on the removal of bromsulfalein from the blood has been widely studied. Early comparisons of the results of determinations of amounts of retention of bromsulfalein and bilirubin in the blood of patients and experimental animals with obstructive jaundice suggested a roughly parallel relationship.[11,12] In contrast, studies of patients with other diseases of the liver or biliary tract with or without hyperbilirubinemia indicated an apparent dissociation between the capacities of the liver to excrete bromsulfalein and bilirubin. It is now well known that a considerable divergence between the results of these tests may exist in a wide variety of conditions which may affect the liver.[13]

The use of these tests of hepatic function in diagnosis and prognosis in patients with viral hepatitis has revealed a variability of pattern in becoming positive or negative at different stages of disease. Thus, it has been shown that the retention of bromsulfalein is often the first test to become positive in the acute phase of disease, indicating hepatic injury before clinical jaundice occurs or before the serum bilirubin is increased above normal. In convalescence the disappearance of clinical jaundice and the return to normal levels of serum bilirubin are usually associated with a normal amount of retention of bromsulfalein in the blood.[14] However, numerous instances of disagreement in results of these tests have also been described in this phase of disease. A normal amount of retention of bromsulfalein in the presence of hyperbilirubine-

mia may occur and, conversely, the persistent retention of abnormal amounts of bromsulfalein in the blood when the total amount of bilirubin is within normal limits is not uncommon and actually is frequently the only evidence of residual hepatic injury.[15]

During the past few years the development of quantitative methods of estimation of "direct" and "indirect" bilirubin has made it possible to show that the ratio of "direct" to "indirect" bilirubin may vary widely[8,16] in health and disease. More recently it has been suggested that the determination of the 1′ direct reacting serum bilirubin gives a quantitative expression of the qualitative direct van den Bergh reaction.[9] The exact significance of absolute or relative changes in the prompt 1′ direct serum bilirubin in relation to amounts of the delayed indirect bilirubin is undetermined, although Watson[17] has suggested that the amount of delayed reacting bilirubin measures the degree of retention of bilirubin by the hepatic cells. It is known that in viral hepatitis the prompt 1′ direct reacting serum bilirubin often increases early in disease before there is any increase above normal in total serum bilirubin; in convalescence the prompt 1′ bilirubin frequently diminishes to normal amounts after the total serum bilirubin has become normal. In small groups of patients, it has been observed that the retention of bromsulfalein became *abnormal* earlier in the acute phase and *normal* earlier in the convalescent phase than the amounts of 1′ direct serum bilirubin.[14] Correlation of large numbers of results of determinations of these tests throughout the course of disease is not available. In the following section the data obtained from study of several hundred patients with viral hepatitis are presented.

Materials and Methods

A group of 956 patients (Group IV) was used to determine the relationship between varying degrees of bilirubinemia and retention of bromsulfalein. All of these patients were beyond the acute phase of viral hepatitis, in periods of disease ranging from the 5th to the 44th week.

Seventeen hundred sixty-five determinations of the amounts of bilirubin (1′ direct and total) and retention of bromsulfalein were made on the serums of 956 patients according to the methods previously described. In all instances the three tests were done in the morning of the same day within two hours of obtaining the serum. The determinations of the serum bilirubin were done in the fasting state. The amount of retention of bromsulfalein was measured sixty to ninety minutes postcibum.

Results

1′ Direct Bilirubin. Comparison of the amounts of 1′ direct bilirubin and degree of retention of bromsulfalein in the same serums indicated that there was apparently no constant quantitative relationship between the results of the two tests. Only 61.7 per cent of 737 serums containing normal amounts of 1′ direct bilirubin (0.2 mg. or less) had normal retention of bromsulfalein, while 55.3 per cent of 1,028 serums containing increased amounts of 1′ direct bilirubin (0.21 to 1.0 mg.) had normal retention of dye. However, a degree of correlation was noted which was of value in making it possible to predict the likelihood of a normal amount of retention of bromsulfalein at various levels of 1′ direct bilirubin. (Table III.) The percentage of serums having normal amounts of retention of dye diminished gradually as the amount of 1′ direct bilirubin increased above 0.3 mg. Thus 62.4 per cent of serums with amounts of 0.3 mg. or less of 1′ direct bilirubin had a normal amount of retention of bromsulfalein; as the amount of 1′ direct bilirubin rose above 0.3 mg., the percentage of serums having normal dye retention declined; and when the level of 1′ direct bilirubin ranged from 0.51 to 1.0 mg., only one out of three serums had a normal percentage of retention of dye. In addition, it was observed that the percentage of strongly positive dye retention tests also increased with larger amounts of bilirubin. Thus only 2.6 per cent of serums with 1′ direct bilirubin under 0.2 mg. had retention of dye

in amounts of 15 to 40 per cent, while 11.2 per cent of serums with 1′ direct bilirubin ranging from 0.51 to 1.0 mg. had strongly positive dye retention tests.

Total Serum Bilirubin. Comparison of the amounts of total bilirubin and degree of retention of bromsulfalein in the same serums indicated also that there was no constant quantitative relationship between the results of these two tests. Only 63.9 per cent of 773 serums with normal amounts of total bilirubin (0.8 mg. or less) had normal retention of dye, while 54.1 per cent of 981 serums with increased amounts of total bilirubin (0.81 to 3.0 mg.) had normal retention of dye. It was also evident, however, that the percentage of serums having normal amounts of retention of bromsulfalein diminished gradually as the amount of total bilirubin increased above 0.8 mg./100 ml. (Table IV.) When the total bilirubin was between 0.8 and 1.4 mg., 57 per cent of serums had a normal amount of retention of bromsulfalein. In serums with amounts of total bilirubin ranging from 1.41 to 3.0 mg., only 38.9 per cent had normal retention of dye. The percentage of strongly positive tests also increased as the total serum bilirubin rose. Thus only 1.6 per cent of serums with total bilirubin less than 0.8 mg. had strongly positive dye retention tests, while 7 per cent of serums with total bilirubin ranging from 1.41 to 3.0 mg. had strongly positive tests.

TABLE III

DETERMINATIONS OF 1′ DIRECT BILIRUBIN AND RETENTION OF BROMSULFALEIN IN THE SERUMS OF 956 PATIENTS IN THE CONVALESCENT PHASE OF VIRAL HEPATITIS

1′ Direct Bilirubin mg./100 ml. Serum	No. of Serums	Percentage of Bromsulfalein Retained		
		0–5	5.1–15	15.1–40
		Per cent of Serums	Per cent of Serums	Per cent of Serums
0–0.20	737	61.7	35.7	2.6
0.21–0.30	412	63.6	33.5	2.9
0.31–0.40	376	56.1	37.5	6.4
0.41–0.50	106	48.1	46.2	5.7
0.51–1.00	134	33.6	55.2	11.2
1.01–1.56	9	44.4	33.3	22.2

TABLE IV

DETERMINATIONS OF TOTAL BILIRUBIN AND RETENTION OF BROMSULFALEIN IN THE SERUMS OF 956 PATIENTS IN THE CONVALESCENT PHASE OF VIRAL HEPATITIS

Total Bilirubin mg./100 ml. Serum	No. of Serums	Percentage of Bromsulfalein Retained		
		0–5	5.1–15	15.1–40
		Per cent of Serums	Per cent of Serums	Per cent of Serums
0.10–0.50	130	63.1	36.2	0.8
0.51–0.80	643	64.4	33.7	1.9
0.81–1.00	424	59.7	33.7	6.6
1.01–1.40	400	54.2	40.5	5.2
1.41–3.00	157	38.9	54.1	7.0

Ratio between 1′ Direct and Total Serum Bilirubin. It is known that in viral hepatitis the 1′ direct serum bilirubin frequently increases above normal before the total serum bilirubin in the acute phase of disease, and subsequently remains increased above normal when the latter has returned to normal in convalescence. The relationship between these two forms of bilirubin in the serums of patients between the 5th and 44th weeks of disease is recorded in Table V.

No exact quantitative relationship was observed between the ratio of 1′ direct bilirubin/total bilirubin and the percentage of retention of bromsulfalein in the blood. Analysis of the groups of serums with abnormal retention of dye indicated that regardless of the amount of total bilirubin (within a range of 0.1 to 1.0 mg.), the percentage of positive dye retention tests was constant within a given range of 1′ direct bilirubin. Thus when the 1′ direct bilirubin ranged from 0 to 0.3 mg., 35.4 to 37.2 per cent of serums had positive dye retention tests; and when the 1′ direct bilirubin ranged from 0.31 to 0.5 mg. at comparable levels of total bilirubin, approximately 45

per cent of serums had positive dye retention tests. These data suggest that when the total bilirubin is under 1.0 mg., the amount of 1′ direct bilirubin in the serum is more closely correlated with the percentage of retention of dye. That this relationship is not constant is indicated, however, by the fact that for comparable levels of 1′ direct bilirubin at levels of total serum bilirubin ranging from 1.0 to 3.0 mg., the percentage of positive bromsulfalein retention tests may increase.

TABLE V

RELATIONSHIP OF AMOUNTS OF TOTAL BILIRUBIN TO THE 1′ DIRECT BILIRUBIN AND ABNORMAL BROMSULFALEIN RETENTION TESTS IN THE SERUMS OF 956 PATIENTS IN THE CONVALESCENT PHASE OF VIRAL HEPATITIS

Total Bilirubin mg./100 ml. Serum	No. of Serums	Serums with Abnormal BSP Tests Per cent	Range of 1′ Direct Bilirubin/100 ml. Serum in Which Abnormal Retention of Bromsulfalein Occurred					
			0–0.30		0.31–0.50		0.51–1.00	
			No. of Serums	Per cent Abnormal	No. of Serums	Per cent Abnormal	No. of Serums	Per cent Abnormal
0.10–0.50	130	36.9	129	37.2	1	0	0	0
0.51–0.80	635	36.1	593	35.4	42	45.2	0	0
0.81–1.00	424	40.1	268	36.6	153	45.8	3	66.7
1.01–3.00	557	50.1	149	45.6	284	45.4	124	66.1

Correlation of Results of Determinations of Retention of Bromsulfalein and Amounts of Serum Bilirubin in Various Weeks of Disease. The results heretofore described were compiled from tests made on serums obtained from the 5th to the 44th week of disease of patients with viral hepatitis. Since it is known that abnormal retention of bromsulfalein often occurs earlier than increase of the serum bilirubin in the acute phase of disease, a comparison was made of the results of similar determinations in the first ten days of viral hepatitis experimentally induced in volunteers.* When the amounts of retention of bromsulfalein were compared with the amounts of total bilirubin in the serums in various periods of disease, a regular pattern of relationship was observed. (Table VI.) In general, the percentage of serums with abnormal retention of dye within a wide range of total serum bilirubin (0.2 to 3.0 mg.) tended to decrease during convalescence until the end of the third month of disease, declining from 76 per cent of serums with abnormal retention in the first ten days of disease to 38.2 per cent of serums with abnormal retention in the third month. During the fourth month the percentage of serums with abnormal retention of bromsulfalein increased somewhat, and observation of a small number of patients over a period of eleven months revealed a similar increase in percentage of abnormal dye retention tests. When the percentage of abnormal dye retention tests was compared with more closely defined ranges of serum bilirubin (1′ direct and total), a similar pattern was observed, although the variation between the second and third month of disease was not noticeable when the amounts of bilirubin were normal. (Tables VII and VIII.)

* These experiments were conducted from 1943–1946 by the Neurotropic Virus Disease Commission, Army Epidemiological Board, U. S. Army, and were recently reviewed.[18]

COMMENT

The data presented in the first section of this paper indicate that patients with viral hepatitis and, to a lesser degree, normal persons have an increased capacity to remove bromsulfalein from the blood when the test is performed postcibum as compared to results obtained in the fasting state.

TABLE VI

ABNORMAL BROMSULFALEIN RETENTION TESTS IN VARIOUS PHASES OF VIRAL HEPATITIS IN 986 PATIENTS WHOSE TOTAL BILIRUBIN RANGED FROM 0.2 TO 3.0 MG./100 ML. SERUM

Days of Disease		Weeks of Disease							
1–10		5–8		9–12		13–16		17–44	
Serums*		Serums		Serums		Serums		Serums	
Tested No.	Abnormal %	Tested No.	Abnormal %	Tested No.	Abnormal %	Tested No.	Abnormal %	Tested No.	Abnormal %
50	76	797	43.3	615	38.2	226	47.8	116	44.0

* These serums were from thirty volunteers with experimentally induced viral hepatitis.[18]

TABLE VII

RELATIONSHIP OF AMOUNTS OF 1′ DIRECT BILIRUBIN TO ABNORMAL BROMSULFALEIN RETENTION TESTS IN THE SERUMS OF 956 PATIENTS DURING CONVALESCENCE FROM VIRAL HEPATITIS

1′ Direct Bilirubin mg./100 ml. Serum	Weeks of Disease					
	5–8		9–11		12–15	
	No. of Serums	Serums with Abnormal BSP Tests Per cent	No. of Serums	Serums with Abnormal BSP Tests Per cent	No. of Serums	Serums with Abnormal BSP Tests Per cent
0–0.30	413	35.6	354	34.5	249	40.6
0.31–0.50	291	47.1	136	40.4	45	46,7
0.51–1.00	101	68.3	20	65.0	6	50.0

TABLE VIII

RELATIONSHIP OF AMOUNTS OF TOTAL BILIRUBIN TO ABNORMAL BROMSULFALEIN RETENTION TESTS IN THE SERUMS OF 956 PATIENTS DURING CONVALESCENCE FROM VIRAL HEPATITIS

Total Bilirubin mg./100 ml. Serum	Weeks of Disease					
	5–8		9–11		12–15	
	No. of Serums	Serums with Abnormal BSP Tests Per cent	No. of Serums	Serums with Abnormal BSP Tests Per cent	No. of Serums	Serums with Abnormal BSP Tests Per cent
0.10–0.80	269	35.3	236	33.9	172	36.0
0.81–1.00	199	40.2	126	34.9	70	45.7
1.01–1.40	233	47.6	107	39.3	48	50.0
1.41–3.00	96	61.5	41	58.5	17	64.7

Although the amount of flow of hepatic blood was not measured in these patients, it is known to be increased following the ingestion of food, and it is suggested that this mechanism may account for the diminished retention of dye in the blood following the meal. The reason for the apparently greater improvement in capacity to clear the blood of dye postcibum by patients with viral hepatitis as compared with normal persons is not known. The possibility exists that impaired flow of hepatic blood is a more common and important factor than heretofore recognized in viral hepatitis. If this is true, the increase in hepatic blood flow produced by ingestion of food might be sufficient to account for the greater increment of improvement in these patients as compared with normal persons. Since the measurement of retention of bromsulfalein is widely used to determine residual hepatic damage following acute hepatitis, it is important to perform the test in the fasting state since a certain number of persons who actually still have an impaired mechanism for removal will not be detected if tested after a meal.

The data presented in the second section of this paper are in agreement with the results of others and indicate a certain degree of correlation between the capacity of the liver to excrete bromsulfalein and bilirubin, although a wide dissociation between these two functions is evident. It is of interest that the degree of correlation between these functions apparently varies with the stage of disease, with greater dissociation in the acute and late convalescent phases. This is in keeping with previous observations that the retention of bromsulfalein frequently becomes abnormal before the serum bilirubin is increased above normal, and may remain abnormal long after the serum bilirubin has become normal, thereby constituting in some instances the only evidence of residual hepatic injury.

The fact that either impaired or normal capacity to remove bromsulfalein from the blood may be present in patients with either normal or increased amounts of serum bilirubin makes it difficult to determine whether there is any mechanism of preferential excretion of these two substances involved or whether this divergence merely represents an association of functional disabilities. Experimentally, Cantarow et al.[5] have shown that there is no evidence of competition between bromsulfalein and bilirubin for a common excretory mechanism at normal concentrations of serum bilirubin. In contrast, these workers suggested that, at elevated concentrations of serum bilirubin, the bromsulfalein and bilirubin may compete for a common excretory mechanism, with the dye being excreted preferentially under these circumstances. The dissociation in the capacity to excrete these two substances in the patients reported here, particularly as observed in patients with normal amounts of bilirubin, suggests that some mechanism other than preferential excretion may condition the difference. The difference in amount of disagreement between the results of the two tests in various phases of disease adds support to this concept.

It is of interest that within the normal range of total serum bilirubin (<0.81 mg./100 ml. serum) there was apparently a degree of correlation between various amounts of 1′ direct bilirubin and the retention of bromsulfalein. It is not known whether this indicates a closer relationship between the individual capacity to excrete the dye and 1′ bilirubin, but the results suggest that knowledge of the amount of 1′ bilirubin in the serum gives a somewhat greater possibility of predicting whether or not the excretion of dye will be normal.

SUMMARY

1. The effect of ingestion of food on the removal of bromsulfalein from the blood was investigated in ninety-two patients with viral hepatitis and twenty-six normal persons. The patients with hepatitis and, to a lesser degree, the normal persons had an increased capacity to clear the dye from the blood when the test was performed sixty to ninety minutes postcibum as compared

with results obtained in testing the same subjects in the fasting state.

2. The relationship between different amounts of bilirubin in the serum (1′ direct and total) and the percentage of retention of intravenously injected bromsulfalein was studied in 986 patients with viral hepatitis.

3. Although there was no constant quantitative relationship between the percentage of retention of dye and the amounts of 1′ direct and total bilirubin, there was a degree of correlation sufficient to make it possible to predict the likelihood of a normal amount of retention of dye at various levels of bilirubin; 61.7 per cent of serums in which the 1′ direct bilirubin measured 0.2 mg. or less had normal retention of bromsulfalein, while only one out of three serums with 1′ direct bilirubin measuring from 0.51 to 1.0 mg. had a normal percentage of retention of dye. When the total bilirubin was normal (0.8 mg. or less) 63.9 per cent of serums tested had normal retention of dye, while only 38.9 per cent of serums with total bilirubin ranging from 1.4 to 3.0 had normal retention of dye.

4. The dissociation in the capacities of patients with hepatitis to remove bromsulfalein and bilirubin from the blood varied somewhat with the phase of disease. In general, within a given range of serum bilirubin the percentage of serums with abnormal retention of dye was higher in the acute phase of disease, tending to decrease during convalescence until the end of the third month. Observation of a small number of patients over a period of several months of protracted convalescence revealed an increase in the number of serums with abnormal retention of dye at the same levels of bilirubin. These data are in accord with previous observations that abnormal retention of bromsulfalein frequently occurs before the serum bilirubin is increased early in disease and, at times, remains as the only evidence of residual hepatic damage in patients whose serum bilirubin has become normal during prolonged convalescence.

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Comparison of the Thymol, Cephalin-cholesterol Flocculation and Colloidal Red Tests in Acute Viral Hepatitis*

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THE thymol turbidity, thymol flocculation, cephalin-cholesterol flocculation and colloidal red tests have been widely used as aids in the diagnosis and evaluation of hepatic injury in viral hepatitis and other diseases of the biliary tract. Extensive data concerning these tests, applied individually or as a group, have been accumulated from the study of a large series of cases of viral hepatitis at the U. S. Army Hepatitis Center, 120th Station Hospital, Bayreuth, Germany. Analysis of these data provided information on the following questions concerning these tests in this disease:

(1) For the thymol test, Mateer and his associates[1] recommended the use of a reagent buffered at a pH of 7.55 rather than that described by Maclagan[2] which utilized a thymol barbiturate reagent buffered at a pH of 7.8. In their experience the thymol reagent buffered at a pH of 7.55 was a more sensitive indicator of hepatic disturbance. With regard to these two variations, which test provides the more useful information for routine purposes? (2) Of the four tests (thymol turbidity, thymol flocculation, cephalin-cholesterol flocculation and colloidal red), which when considered alone produces the greatest number of positive responses? (3) Does the use of two or more of these tests provide more information than the use of any one test alone? If so, which combination of tests gives the greatest number of positive responses? (4) In what percentage of cases of viral hepatitis are all of these tests within the normal range throughout the course of the disease?

MATERIALS AND METHODS

The data used in this analysis were obtained from studies on patients with clinically diagnosed viral hepatitis admitted to the Hepatitis Center between August 11, 1947, and October 15, 1948. The patients were predominantly soldiers in the U. S. Army, the majority being between the ages of eighteen and twenty-five.

The thymol turbidity tests were done with reagents buffered at pH 7.8 and pH 7.55. Reagents were prepared and the tests performed according to the method of Maclagan.[2] The turbidities were measured in a Coleman Junior Photocolorimeter, using a barium sulfate standard as recommended by Shank and Hoagland.[3] The results were expressed in Shank-Hoagland units. The thymol flocculation, described by Neefe,[4] was also read in association with the thymol turbidity tests. The cephalin-cholesterol flocculation was done according to the method of Hanger,[5] using Difco antigen with modifications described by Neefe and Reinhold.[6] The colloidal red test was performed by the method of Ducci.[7]

* From the University of Pennsylvania School of Medicine and the 120th Station Hospital (Hepatitis Research Team), European Command, U. S. Army of Occupation in Germany. Representing work done under the auspices of the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, Office of The Surgeon General, U. S. Army, Washington, D. C. Statistical analysis was aided, in part, by the Commission on Liver Diseases, Armed Forces Epidemiological Board.

Normal Values. The normal ranges for these tests were based on studies carried out at the Hepatitis Center and on data previously obtained at the Hospital of the University of Pennsylvania.[8] Table I presents the normal and equivocal ranges, the number of subjects from which these data were obtained and the sources of the data. For each test the mean and the standard deviation were computed. Normal values were considered to be those within two standard deviations from the mean. Values exceeding two standard deviations but not exceeding three standard deviations from the mean were considered as equivocal. All results above three standard deviations were considered to be abnormal (positive).

Method of Analysis. The basic group concerned in this analysis consisted of 1,860 patients with acute viral hepatitis and represented patients for whom thymol turbidity responses with reagent buffered at pH 7.8 were available. In the majority of the group the thymol flocculation response at pH 7.8 was also available. It was not considered necessary to perform all tests herein discussed on all patients throughout the period of this study, and certain tests were discontinued

TABLE I

NORMAL VALUES USED FOR THYMOL TEST, CEPHALIN-CHOLESTEROL FLOCCULATION TEST AND COLLOIDAL RED TEST*

Test	Negative	Equivocal Test	Positive	Subjects
Thymol turbidity pH 7.8	Below 5.8	5.8–6.9	>6.9	260 H.R.C.
Thymol turbidity pH 7.55	Below 7.4	7.4–8.9	>8.9	121 H.R.C.
Thymol flocculation pH 7.55	0, ±	1+	2 to 4+	65 H.U.P.
Thymol flocculation pH 7.8	0, ±	1+	2 to 4+	260 H.U.P.
Cephalin-cholesterol flocculation, 24 hr.	0	±	1 to 4+	175 H.U.P.
Cephalin-cholesterol flocculation, 48 hr.	0, ±, 1+	2+	3 to 4+	175 H.U.P.
Colloidal red	0, ±	1+	2 to 4+	65 H.U.P.

* Source of test described in text. Thymol turbidity expressed in Shank-Hoagland units. Thymol flocculation, cephalin-cholesterol flocculation, colloidal red reactions graded from 0–4+. H.R.C., Hepatitis Research Center, 120th Station Hospital, Bayreuth, Germany; H.U.P., Hospital of the University of Pennsylvania. Last column indicates the number of normal subjects from which these data were derived.

TABLE II

TOTAL NUMBER OF PATIENTS AND TOTAL NUMBER OF OBSERVATIONS AVAILABLE FOR BASIC GROUP*

Test	No. of Patients	No. of Observations	Average No. of Observations
Thymol	1,860	13,053	7.0
Cephalin-cholesterol flocculation	677	4,668	6.8
Colloidal red	688	4,812	7.0

* Last column represents arithmetic average number of observations available for each patient. Thymol test, Group T-1,860; cephalin-cholesterol flocculation test, Group CCF-677; colloidal red, Group CR-688.

TABLE III

NUMBER OF PATIENTS OBSERVED IN EACH WEEK OF DISEASE*

Week	Thymol Turbidity		Thymol Flocculation		Cephalin-Cholesterol	Colloidal Red
	7.8	7.55	7.8	7.55		
1	104	87	102	83	20†	18†
2	684	529	668	515	231	234
3	1,184	936	1,159	917	441	437
4	1,423	1,122	1,396	1,096	545	567
5	1,439	1,101	1,405	1,086	520	528
6	1,466	1,111	1,436	1,101	532	547
7	1,313	975	1,293	968	496	503
8	1,180	848	1,163	839	446	448
9	1,025	649	1,015	685	356	389
10	872	584	864	574	304	327
11	660	438	656	436	236	242
12	497	331	495	326	185	191
13	374	236	366	233	139	135
14	267	175	263	173	92	92
15	181	121	176	119	54	52
16	133	83	132	82	43	49
17	89	55	87	55	28‡	30‡
18	74	40	73	40	.....	23‡
19	54	30‡	53	.....	.....	.....
20	34‡	.....	34‡	.....	.....	.....
Totals	13,053	9,451	12,836	9,328	4,668	4,812
Group	T-1,860	T-1,860	T-1,860	T-1,860	CCF-667	CR-688

* The number of patients is also equivalent to the number of observations available for each test in each week of disease. Week indicates week of disease as defined in text.

† Not included in calculation because of inadequate sampling.

‡ Included in Figures 1, 2 and 3 but statistical significance questionable.

or disregarded after sufficient data for analysis had accumulated.

The total number of patients and the total number of observations for each test by week of disease are presented in Tables II and III. The week of disease was computed for each patient from the date of onset of initial symptoms. Although this was the best available estimate of the stage of disease, the majority of patients had an insidious onset, with the occurrence of bilirubinuria and/or scleral icterus as the initial sign of disease. Thus in all probability alterations in hepatic functions were already present in most of these patients at the time of appearance of signs or symptoms.[9] The designation of "week of disease" should be understood, therefore, to signify the number of weeks after the *recognized* onset of signs or symptoms, the disease possibly being already well established by this time.

It will be noted that fewer patients were observed during the first two weeks of disease, particularly the first week, than during the following weeks. This was due to several factors, namely, (1) failure of patients to report at the onset of illness and/or delay in diagnosis and (2) difficulty in transportation which prevented transfer of some patients to the Hepatitis Center before the lapse of the second week of disease. The decreasing number of observations after the twelfth week of disease reflects the comparatively few patients with evidence of persistent hepatic disturbance who were still hospitalized.

For the purpose of analyzing the data presented herein it was necessary to divide the patients into several groups. To facilitate reference to these groups each group was designated by letters (T, CCF, CR, Cor) to identify the test or correlation of tests and a number representing the total number of patients in the particular group.

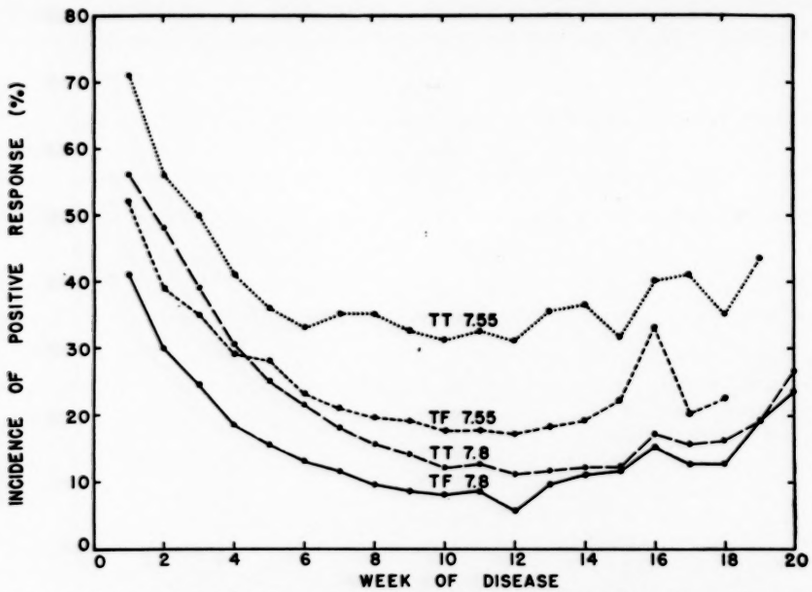

FIG. 1. Group T-1860: The incidence of positive responses by week of disease of the thymol turbidity and flocculation tests performed with reagents buffered at a pH of 7.55 and 7.8 in 1,860 patients with acute viral hepatitis. The number of observations represented in each week of disease for each test is noted in Table III; week of disease as defined in text. Increased incidence after twelfth week probably due to factor of selection. TT and TF represent thymol turbidity and thymol flocculation at buffers indicated.

Thymol Tests. The analysis of the data on the thymol tests was accomplished in several ways:

I. The incidence of positive responses (for the thymol turbidity and thymol flocculation tests at pH 7.8 and pH 7.55) at different stages of disease was computed by determining the percentage of positive responses in each week of disease. The basic number of cases so analyzed is presented in Table III, and the percentage of positive responses in each week of disease is graphically represented in Figure 1 (Group T-1,860). For the purpose of this analysis the thymol turbidity and thymol flocculation tests were considered separately.

II. To obtain additional data concerning these four thymol tests the responses of all patients who

were tested simultaneously with all four tests in at least each of the first four weeks of disease were analyzed. This group consisted of seventy-eight cases (Group T-78). For an additional seven patients only turbidity and flocculation responses with the pH 7.8 buffer were available.

The percentage of positive responses with each of the four thymol tests during the first week of disease was computed. The percentage of patients who were not abnormal on admission but who demonstrated positive responses in any of the succeeding weeks of disease was also computed, and this is noted in Table IV in a cumulative fashion (Group T-78). Moreover, in order to determine the duration of positive thymol responses in this disease the week of disease in which a previously abnormal response reverted to normal was noted for each person; the percentage so represented was also noted in a cumulative fashion. (Table IV.)

III. Since the aforementioned seventy-eight cases are a comparatively small group and the number available during the first week of disease having been limited by factors already mentioned, the data pertinent to all cases which had been observed at least once in the first two weeks of disease and at least three times during the first four weeks of disease were used. Therefore, this analysis included data from 401 patients who were observed during at least weeks one

TABLE IV
GROUP T-78*

Week of Disease	Thymol Turbidity pH 7.8		Thymol Turbidity pH 7.55		Thymol Flocculation pH 7.8		Thymol Flocculation pH 7.55	
	Abnormal Prior to End of Week (per cent)	Subsequently Negative Prior to End of Week (per cent)	Abnormal Prior to End of Week (per cent)	Subsequently Negative (per cent)	Abnormal Prior to End of Week (per cent)	Subsequently Negative (per cent)	Abnormal Prior to End of Week (per cent)	Subsequently Negative (per cent)
1	74.1	N.O.	83.4	N.O.	61.2	N.O.	67.8	N.O.
2	78.6	12.9	85.9	10.3	67.0	7.1	79.5	10.1
3	80.0	32.9	85.9	16.7	71.7	23.5	82.5	17.9
4	80.0	43.5	85.9	38.5	71.7	38.8	82.0	29.5
5		49.4		41.0		43.5		35.9
6		63.5		47.4		40.0		41.0
7		63.5		51.3		54.1		43.5
8		67.0		51.3		54.1		47.4
Total abnormal (per cent).....	80.0		85.9		74.7		82.0	
No determination possible (per cent).....		7.1		15.4		7.1		7.7
Total no. observed	85		78		85		78	

* Incidence of positive responses of thymol turbidity and flocculation tests at pH 7.8 in 78 patients plus an additional 7 patients tested at pH 7.8 observed once in at least each of first 4 weeks of disease in terms of cumulative percentage. N.O.: no observations. No determination possible: patients not observed in succession of weeks past 4th week and therefore exact week on which abnormal responses returned to normal not determinable.

TABLE V
GROUP T-401*

Type of Response, Test	Positive	Equivocal	Negative	Total
Thymol turbidity pH 7.55..	238	62	101	401
Thymol turbidity pH 7.8...	209	63	129	401
Thymol flocculation pH 7.55	170	97	134	401
Thymol flocculation pH 7.8.	144	83	174	401

* Number of patients demonstrating on admission positive, equivocal or negative (normal) turbidity and flocculation tests at buffer pH 7.8 and buffer pH 7.55 in 401 patients with acute viral hepatitis.

and/or two and weeks three and four (Group T-401).

(1) The incidence of positive responses of the thymol test on admission in this group of 401 cases is shown in Table v (Group T-401). (2) patients who demonstrated persistently normal responses throughout the course of disease as determined by any one of the four thymol tests and by various combinations of these tests is shown in Table viii (Group T-401).

TABLE VI
GROUP T-401*

Test		Thymol Turbidity pH 7.8			Test		Thymol Flocculation pH 7.8		
		N	E	P			N	E	P
Thymol	N	72	1	0	Thymol	N	84	2	0
Turbidity	E	29	26	1	Flocculation	E	50	57	0
pH 7.55	P	12	25	235	pH 7.55	P	2	37	169

* Comparison of responses of thymol turbidity at pH 7.55 and pH 7.8 and thymol flocculation at pH 7.55 and 7.8 concomitantly determined in 401 cases of acute viral hepatitis. A positive result is indicated for each test if a patient demonstrated an abnormal response in the particular test at any time during the course of disease. N, normal response: E, equivocal response; P, positive response.

The results of the thymol turbidity test at pH 7.8 were compared with the thymol turbidity test at pH 7.55; the flocculation tests with the respective buffers also were compared. (Table vi, Group T-401.) Any patient who had a positive response with the particular test concerned at any time during the course of disease was considered as falling into the abnormal category for that particular test. Thus the number of negative, equivocal or positive responses with the various tests during the course of disease was compared. (3) The thymol test which first yielded an abnormal response in each patient was noted; the tabulated totals are recorded in Table vii (Group T-401). For this purpose if more than one test exhibited an abnormal response at the same time, tabulations were made to include both tests. (4) Since a significant number of patients had normal responses to one or more of these tests, further evaluation of the relationship between the four thymol tests was attempted by correlation. The percentage of

TABLE VII
GROUP T-401*

Test	No. of Patients
Thymol turbidity pH 7.55	243
Thymol turbidity pH 7.8	205
Thymol flocculation pH 7.55	160
Thymol flocculation pH 7.8	143

* The number of instances in 401 patients in which each thymol test was either the first to denote an abnormal response or became positive simultaneously with one of the other thymol tests. In cases in which more than one test was abnormal simultaneously each test is recorded.

Cephalin-cholesterol Flocculation Test. (For the purpose of this analysis, the twenty-four- and forty-eight-hour readings were considered independently.) The percentage of positive responses was computed in each week of disease for 667 cases observed 4,614 times during the course of the disease. The distribution by week of disease is recorded in Table iii and is graphically represented in Figure 2 (Group CCF-667).

TABLE VIII*
GROUP T-401*

Test or Combination	No. of Patients with Normal Responses	Percentage of Normal Responses
TT—7.8	114	28
TF—7.8	137	32
TT—7.55	73	18
TF—7.55	86	21.5
TT—7.8 and TT—7.55	72	18
TF—7.8 and TF—7.55	84	21
TT—7.8 and TF—7.8	98	24.5
TT—7.55 and TF—7.55	60	15
All tests	60	15

* Incidence in 401 patients of normal responses with thymol turbidity and thymol flocculation tests (using reagents buffered at pH 7.55 and pH 7.8) throughout the course of acute viral hepatitis. All tests—thymol turbidity pH 7.55 and pH 7.8 and thymol flocculation at pH 7.55 and pH 7.8. TT, thymol turbidity; TF, thymol flocculation.

Colloidal Red Test. The percentage of positive responses was computed in each week of disease for 688 patients observed 4,820 times during the course of the disease. The distribution by week of disease is represented in Table 1 and the percentage of positive responses is recorded in Figure 3 (Group CR-688).

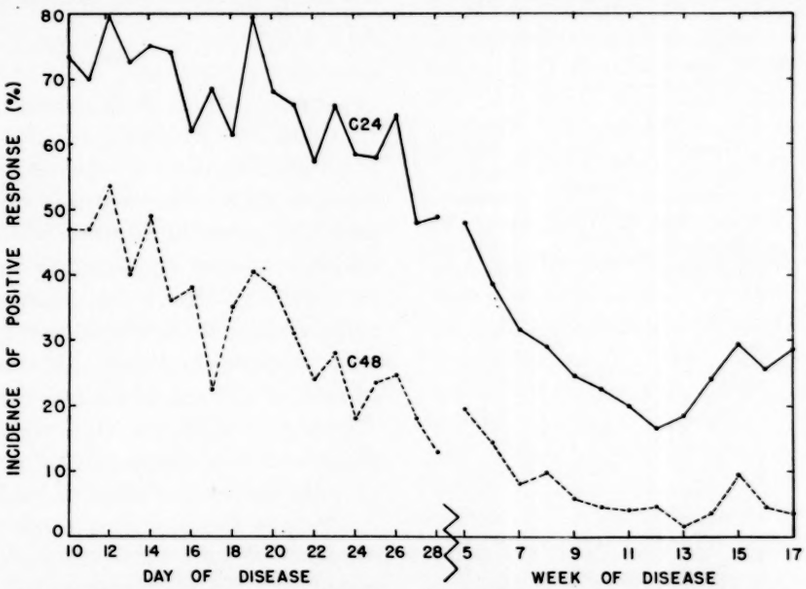

FIG. 2. Group CCF-667: The incidence of positive responses by days of disease for first twenty-eight days and week of disease thereafter of the cephalin-cholesterol flocculation test in 667 patients with acute viral hepatitis. The twenty-four- (C-24) and forty-eight- (C-48) hour readings are considered independently. The apparent discrepancy between C-24 and C-48 hour readings is principally due to the fact that a 1+ reading at twenty-four hours was considered positive whereas readings less than 2+ at forty-eight hours were considered negative; week of disease as defined in text.

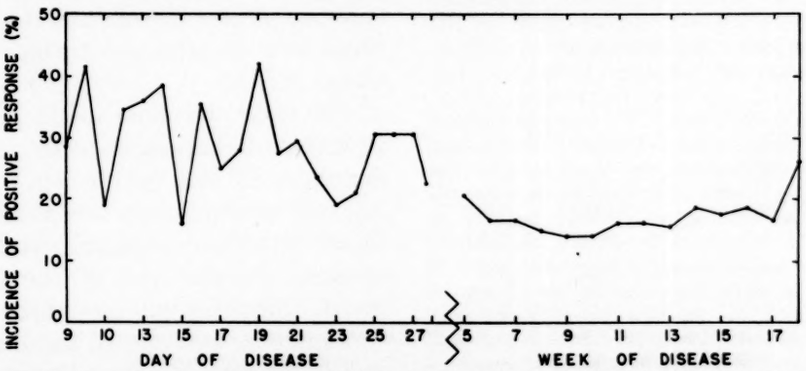

FIG. 3. Group CR-688: Incidence of positive responses of colloidal red test (by days of disease for first twenty-eight days and weeks of disease thereafter) in 688 patients with acute viral hepatitis; week of disease as defined in text.

Correlation of Tests. For the purpose of observing the character of the response of any one test when compared with any or all of the other tests, the data were analyzed on all cases which had had concomitantly the thymol turbidity test at pH 7.55, the thymol flocculation test at pH 7.55, the cephalin-cholesterol flocculation test and the colloidal red test, and which had been studied at least once in the first two weeks and three times in the first four weeks of disease. As will be noted later the thymol tests utilizing reagent buffered at pH 7.55 were selected for this correlation as this reagent was shown to be more sensitive than the buffer at pH 7.8. Responses of the cephalin-cholesterol flocculation

tests were considered positive if either or both of the twenty-four- and forty-eight-hour readings were positive.

Suitable data were available for 108 patients (Group Cor-108). The pattern of response of the four tests (thymol turbidity at pH 7.55, thymol flocculation at pH 7.55, cephalin-cholesterol flocculation and colloidal red) on admission is recorded in Column 2, Table IX. A positive response is considered to have occurred if any or all tests were positive on admission in each particular combination. The pattern of response when all of the four tests were positive at some time during the course of disease is recorded in Column 3. The pattern of response when any of the tests in each particular combination were positive at some time during the course of the disease is recorded in Column 4.

TABLE IX
GROUP COR-108*

	Positive on Admission. (Any Test Positive) (per cent)	All Tests Simultaneously Positive (per cent)	Positive Sometime during Disease (Any Test in Combination) (per cent)
TT, TF	61.1	36.1	68.5
TT, TF, CCF	82.4	36.1	84.3
TT, TF, CCF, CR	82.4	22.2	84.3
TT, TF, CR	62.0	22.2	71.3
TT, CCF	82.4	56.5	84.3
TT, CR	61.1	27.8	70.4
TT, CCF, CR	82.4	26.9	84.3
TF, CCF	79.6	38.0	82.4
TF, CR	44.4	23.2	52.8
TF, CCF, CR	80.6	23.2	83.3
CCF, CR	80.6	28.7	84.3
TT	60.2	....	66.7
TF	38.0	....	45.4
CCF	79.6	....	81.5
CR	30.5	....	44.4

* Correlation of Thymol turbidity pH 7.55; thymol flocculation pH 7.55; cephalin-cholesterol flocculation and colloidal red tests observed concomitantly in 108 cases of acute viral hepatitis. Figures represent percentage of positive responses in the 108 cases. Column 1: TT, thymol turbidity; TF, thymol flocculation; CCF, cephalin-cholesterol flocculation; CR, colloidal red test listed in all possible combinations. Column 2: Percentage of cases positive on admission in any or all tests of particular combination. Column 3: Percentage in which all tests in combination were simultaneously positive at some time during the course of the disease. Column 4: Percentage in which at least one of a particular group of tests was positive at some time during the course of disease. Seventeen (15.7%) patients demonstrated normal responses throughout course of disease in these tests. All positive cephalin-cholesterol flocculation responses were present by end of 4th week. All but 2 of positive thymol turbidity responses were present by end of 4th week.

RESULTS

Thymol Test. Regardless of the various methods of analysis of the data on the thymol test, the results uniformly demonstrated that the thymol turbidity test at pH 7.55 gave the highest number of positive responses, and the thymol flocculation test at pH 7.8 the least number of positive responses. The highest percentage of positive responses (with all four tests) was obtained during the first week of disease. The limiting factors concerning the designation "week of disease" and should be borne in mind in respect to this point.

During the *first four weeks* of disease the analysis of the four thymol tests on the basic group of 1,860 patients (Group T-1,860) indicated that the two thymol turbidity tests (pH 7.55 and 7.8) gave a greater number of positive responses than did either of the respective thymol flocculation tests. However, during the succeeding weeks of disease the thymol flocculation at pH 7.55 gave a greater number of positive responses than did the thymol turbidity at pH 7.8. (Fig. 1.)

Of those patients with positive thymol reactions who were observed at weekly intervals (Table IV, Group T-78) in at least each of the first four weeks of disease, more than half had demonstrated positive responses by the end of the first week. Of these, all positive responses were still in evidence by the end of the third week. This holds true for the four thymol tests. By the end of the eighth week significantly fewer patients had reverted to normal limits when evaluated by the thymol test at pH 7.55 than had reverted to normal limits with the thymol turbidity at pH 7.8.

Of the four variations of the thymol test, the thymol turbidity at pH 7.55 not only gave the greatest number of positive re-

sponses (Table v, Table vi, Group T-401) during the course of the disease but also was the first to exhibit an abnormal reading in the majority of patients. (Table vii, Group T-401.) The thymol test at pH 7.8 was never abnormal when the thymol test at pH 7.55 was normal. (Table vi, Group T-401.) The converse was not true. This applies to both the thymol turbidity and thymol flocculation tests.

A significant number of patients had consistently negative thymol reactions throughout the course of the disease when evaluated by the four thymol tests (15 per cent of 401 patients). (Table viii, Group T-401.) The percentage of patients which remained consistently negative throughout the course of the disease varied with the test or combination of tests utilized. If the thymol flocculation test at pH 7.8 is considered alone, 32 per cent of 401 patients exhibited normal responses throughout the course of disease. When the thymol turbidity at pH 7.55 and thymol flocculation at pH 7.55 were used as the only criteria of hepatic disturbance, only 15 per cent of the patients failed to reveal abnormal responses at some time during the disease.

Cephalin-cholesterol Flocculation Test. The greatest number of positive responses with the cephalin-cholesterol flocculation test was obtained at the end of the second week. (Fig. 2, Group CCF-667.) A sharp rise was present on the nineteenth day, but no explanation is apparent for this finding. The twenty-four-hour readings yielded a greater number of positive responses than did the forty-eight-hour readings, principally due to the fact that a 1+ positive response at twenty-four hours was considered to be positive whereas a 1+ response at forty-eight hours was considered negative. The rise in percentage of positive responses after the twelfth week is attributable to the fact that the patients who had not experienced a prolonged or complicated disease had been discharged from the hospital by this time.

Colloidal Red Test. The colloidal red test demonstrated positive responses in less than 40 per cent of the patients during the first twenty-eight days (38 to 18 per cent) except the eleventh and eighteenth days (Fig. 3, Group CR-688), and the variation on these two days is not significant. The percentage of positive responses of the colloidal red test is significantly less than the positive responses of the thymol turbidity test (70 to 30 per cent) and the cephalin-cholesterol flocculation test (80 to 50 per cent) during this period. After the first four weeks, it remained at a constant level (15 to 18 per cent) even after the twelfth week of disease, in contrast to the higher incidence of positive responses with both the thymol and cephalin-cholesterol flocculation tests.

Correlation of Tests. Of the 108 patients who fulfilled the criteria previously mentioned (Group Cor-108), seventeen (15.7 per cent) had negative responses with all tests herein considered throughout the course of the disease. (Table ix.) Two patients (1.9 per cent) who revealed normal responses on admission became positive at a later date. By the end of the second week 79.6 per cent of the patients had positive responses with the cephalin-cholesterol flocculation test whereas 60.2 per cent were positive with the thymol turbidity at pH 7.55. When the cephalin flocculation and thymol turbidity tests were considered together, the percentage of positive responses was 82.4 per cent. This is due to the fact that three patients who responded negatively throughout the course of the disease in the cephalin-cholesterol flocculation test revealed positive responses with the thymol turbidity test.

The percentage of positive responses in this series does not increase when, in addition to the thymol turbidity at pH 7.55 and cephalin-cholesterol flocculation tests, the thymol flocculation and colloidal red tests are employed. All positive cephalin-cholesterol flocculation tests were positive by the end of the fourth week of disease. With the exception of two cases, all positive thymol turbidity responses were also positive by the end of the fourth week. The positive responses of the thymol flocculation and

colloidal red tests were significantly increased after the second week of disease.

COMMENTS

Analysis of the thymol responses demonstrates clearly that the use of a thymol reagent buffered at pH 7.55 is associated with a higher incidence of positive responses in acute viral hepatitis than with the reagent buffered at pH 7.8. This supports the contention of Mateer and his co-workers[1] that the thymol test at pH 7.55 is a more sensitive indicator of hepatic disturbance. It does not, however, necessarily mean that the thymol test buffered at pH 7.55 is superior in all respects to the pH 7.8 reagent. As Mateer originally pointed out, the greater sensitivity of the pH 7.55 thymol buffer has its main advantage when detection of minimal or subclinical hepatic disturbance is the objective. However, this increased sensitivity possibly may be a disadvantage when the test is used as an aid in differential diagnosis of jaundice due to intrahepatic disease or extrahepatic obstruction. Thus although the cephalin-cholesterol flocculation test may be positive in cases of extrahepatic biliary obstruction, it is relatively uncommon for the thymol test at pH 7.8 to be positive in this condition.[10] Further study of the buffer at pH 7.55 in this regard and in other types of hepatic disease will be required before the over-all value of the pH 7.55 buffer as compared with the original buffer can be defined.

The apparent increase in the positive responses of the pH 7.55 thymol flocculation test in comparison with the pH 7.8 thymol turbidity test after the fourth week of disease is noteworthy since it constitutes a reversal of the relative sensitivities of these two tests during the first four weeks. This observation appears to offer some support for the view that the stability of the thymol-barbital-protein aggregation, which is thought to govern the thymol flocculation response, requires an additional factor or factors other than those related to the production of turbidity alone. It is our present opinion that the chief value of the thymol flocculation test is the additional assistance it provides in evaluating the significance (in respect to hepatic disturbance) of high normal, equivocal and certain positive thymol turbidity readings. Thus some instances of falsely positive thymol turbidity readings such as those due primarily to disturbances in lipids may be suggested by an associated negative thymol flocculation reaction. On the other hand, an equivocal turbidity reading associated with strongly positive flocculation affords greater significance to the turbidity reading. Evidence to date indicates that positive thymol flocculation tests are unusual in the absence of factors associated with hepatic disturbance.

The fact that the greatest number of positive responses with the four thymol tests (Group T-1,860, Fig. 1) was obtained during the first week of disease, as defined herein, requires further comment. Previous studies of experimentally induced hepatitis of both varieties (IH and SH) have revealed an interesting difference in mode of onset of disease due to these two causative agents.[9,11] In hepatitis due to virus SH the onset, as observed in volunteers, consistently was insidious, the occurrence of symptoms frequently being preceded by hepatic disturbance demonstrable by hepatic tests for days and occasionally for weeks.[9] This contrasted strikingly with the onset of hepatitis due to virus IH in which the onset of symptoms was well defined and following which evidence of hepatic disturbance demonstrable by hepatic tests often did not develop for three to ten days.[11] Thus, in the case of hepatitis (SH) designation of week of disease based on the time of recognition of clinical symptoms or signs could easily result in erroneous designation of the second or third week of disease as the first week of disease. However, the parallelism of the tests (Fig. 1, Group T-1,860) and the consistency of results support the validity of comparison of the tests on the basis of week of disease as previously defined.

Of the four tests (thymol turbidity at pH 7.55, thymol flocculation at pH 7.55,

cephalin-cholesterol flocculation and colloidal red), when considered individually, the cephalin-cholesterol flocculation test revealed the highest incidence of positive responses (79.6 per cent of 108 cases). (Table IX, Group Cor-108.) Thus cephalin-cholesterol flocculation seemed to be the most sensitive of the tests employed when used as an aid in detecting the presence of parenchymal liver disturbance during the active stage of the disease. The incidence of positive responses during this stage of the disease compared with that obtained by the cephalin flocculation test alone (79.6 per cent) was only slightly increased by employing, in addition, the thymol turbidity test at pH 7.55 (82.4 per cent). However, during the convalescent stage the thymol test, when positive, was superior to the cephalin flocculation test as an aid in demonstrating persistent abnormality (40 per cent for the thymol turbidity test as compared with 20 to 30 per cent for the cephalin-cholesterol flocculation test, Group T-1,860, CCF-667). (Figs. 1 and 2.) This again confirms previous reports of the superiority of the thymol test in this respect, and the over-all findings emphasize the necessity for employing both the cephalin-cholesterol flocculation and the thymol tests together for maximum assistance in the study of hepatic disease.

Also worthy of emphasis is the considerable number of patients with viral hepatitis in whom negative responses with all of these tests were obtained throughout the course of disease (Table VIII, Group T-401), indicating that such negative responses need not afford evidence against the diagnosis. Also of interest are the previous observations of an apparent difference in the incidence of positive thymol responses in Virus IH and Virus SH hepatitis as observed in volunteers.[10,11] Thus in Virus IH hepatitis positive thymol tests were the rule, whereas in Virus SH hepatitis the thymol test was negative or only weakly positive in approximately 50 per cent of the cases. As concerned with the present series of cases, the association of a relatively low over-all incidence of positive thymol tests with a relatively high incidence of the insidious type of onset corresponds more closely with the Virus SH hepatitis syndrome as observed in volunteers than with that of the Virus IH hepatitis syndrome. This might be regarded as evidence suggesting that many of the patients in the present series had the Virus SH type of infection. The results in this series of cases indicate that the use of the colloidal red test in addition to the cephalin-cholesterol flocculation and thymol tests did not provide additional helpful information. Thus there appears to be no need for the routine use of the colloidal red test in addition to the thymol and cephalin-cholesterol flocculation tests in the study of viral hepatitis.

SUMMARY AND CONCLUSION

An analysis is presented of the responses of the thymol tests at pH 7.55 and pH 7.8, the cephalin-cholesterol flocculation test and the colloidal red test as observed in patients with viral hepatitis studied at the Hepatitis Center, 120th Station Hospital, Bayreuth, Germany.

The thymol-barbital reagent buffered at pH 7.55 gave a higher incidence of positive responses at all stages of the disease than the same reagent buffered at pH 7.8. Further definition of the status of the test using the pH 7.55 buffer in other diseases of the liver and biliary tract is needed before the pH 7.8 reagent, now in routine use, is discontinued. The greater sensitivity of pH 7.55 reagent may eventually prove to be a disadvantage when the test is used as an aid in the differential diagnosis of jaundice.

Flocculation of a thymol-barbiturate reagent in viral hepatitis appears to require some factor or factors other than those responsible for turbidity alone. The advantages obtained by routine observation of the flocculation reaction as part of the thymol turbidity test are discussed.

Negative responses throughout the course of the disease with all the tests discussed herein do not afford evidence against the diagnosis of viral hepatitis.

For maximum assistance in the study of hepatic disease the cephalin-cholesterol flocculation test should be used in conjunction with the thymol turbidity and thymol flocculation tests.

When used in addition to the cephalin-cholesterol flocculation test and the thymol turbidity and flocculation tests, the colloidal red test did not provide additional information helpful in the study of viral hepatitis.

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Oral Cholecystography in Patients with Viral Hepatitis*

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THE use of cholecystography following the reception of radio-opaque dyes in jaundiced patients has been generally avoided because of the belief that the procedure is unreliable. It has been thought by many that disease of the liver or extrahepatic biliary tract sufficient to interfere with the excretion of bilirubin might also interfere with the excretion and concentration of radio-opaque dye in the amount required for visualization of the gallbladder radiographically. In addition, the fear of possible toxic effects of certain radio-opaque substances on a damaged liver has discouraged their use. In spite of this rather widespread general opinion, it was clearly shown several years ago that certain patients with jaundice excrete and concentrate such materials. Visualization of the gallbladder by roentgenogram, following the intravenous or oral administration of sodium tetraiodophenolphthalein, was described in a large percentage of two groups of patients with intrahepatic disease including catarrhal jaundice.[1,2] More recently Barker et al.[3] reported that in certain patients with active hepatitis but without clinical jaundice the gallbladder was not visualized following this procedure. Neither these investigators nor others,[4,5] in experimental work on dogs, observed any toxic effect of such dyes when used in the presence of jaundice although occasional untoward effects have been reported.[6,7]

The excretion and concentration of radio-opaque dye in the gallbladder to a degree sufficient to be detected by roentgenogram, following oral or intravenous administration, is one of the measures of function of the liver and extrahepatic biliary system. The present study was undertaken to determine in patients with viral hepatitis the relationship of various degrees of hepatic damage, as measured by certain tests of hepatic function, to the capacity to excrete and concentrate radio-opaque dye in the gallbladder.

MATERIALS AND METHODS

Subjects. Forty-three male patients, aged eighteen to forty-six years, whose period of disease ranged from eleven to seventy-eight days, were used as subjects. All patients were clinically jaundiced but were in the phase of diminishing bilirubinemia. The total serum bilirubin measured from 2.29 mg. per cent to 24.00 mg. per cent, and the one-minute serum bilirubin from 1.0 mg. per cent to 12.4 mg. per cent;[8] the retention of bromsulfalein[9] in the blood (forty-five minutes after the intravenous injection of 5 mg. of dye/kg. of body weight) ranged from 3.75 to 66.0 per cent.

Each patient was given six tablets of priodax®,[10] orally after a light supper (one tablet every five minutes). The more deeply jaundiced patients were given twelve tablets of priodax® initially. Approximately fourteen hours following ingestion of the drug a roentgenogram was made in the fasting state. If dye was visibly concentrated in the gallbladder, a fatty meal was given and another roentgenogram was made one hour later to determine if normal emptying of the gallbladder had occurred. If

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* From the Section of Preventive Medicine, Yale University School of Medicine, and the 120th Station Hospital (Hepatitis Research Team), European Command, U. S. Army of Occupation in Germany. Representing work done under the auspices of the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, Office of The Surgeon General, U. S. Army, Washington, D. C.

the gallbladder was not visualized on the first examination, another roentgenogram was made in three hours. If there was still no visualization, dye was readministered orally that same evening, giving twelve tablets of priodax,® and roentgenograms were made the following morning according to the previous schedule.

TABLE I

AGE, PHASE OF DISEASE, HEPATIC ENLARGEMENT AND TENDERNESS, DEGREE OF BILIRUBINEMIA AND RETENTION OF BROMSULFALEIN IN NINE PATIENTS WITH VIRAL HEPATITIS IN WHOM THE GALLBLADDER WAS NOT VISUALIZED BY CHOLECYSTOGRAPHY FOLLOWING INGESTION OF PRIODAX

Case No.	Age	Day of Disease	Hepatic		Total Serum Bilirubin (mg. %)	Bromsulfalein Retention* (% in 45 min.)
			Enlargement	Tenderness		
6	21	21	0	0	2.43	10.75
19	22	36	0	0	4.80	12.50
26	21	22	+	+	5.60	32.70
37	23	11	0	0	14.16	40.00
39	34	44	+	+	14.16	55.00
40	26	33	+	+	14.40	50.00
41	24	30	+	+	14.00	50.00
42	29	46	0	+	20.60	58.50
43	34	28	+	0	24.00	55.00

*Forty-five minutes after the intravenous injection of 5 mg. dye/kg. body weight.

RESULTS

In thirty-four of the forty-three patients (79 per cent) studied the gallbladder was visualized in roentgenograms made after the ingestion of priodax.® In five of these patients the gallbladder was not visualized after the ingestion of six tablets of priodax® but showed good concentration of dye and emptying of the gallbladder following a fatty meal after the ingestion of twelve tablets of priodax.® Of the nine cases in which the gallbladder was not visualized, one received only a single dose of priodax® (six tablets) but the other eight patients received a double dose of dye orally (twelve tablets of priodax®).

There was no apparent relationship between the capacity to excrete and concentrate the dye and the age of patient or the presence or absence of hepatic enlargement and tenderness. The age and amounts of serum bilirubin and retention of bromsulfalein in the nine patients whose gallbladders were not demonstrable roentgenographically are shown in Table I. Only two were over thirty years of age (both thirty-four) and none of them had any previous history of disease of the biliary tract. The retention of bromsulfalein was 30 per cent or more in seven of these men; six were deeply jaundiced with amounts of total serum bilirubin measuring 11.0 mg. per cent or more. Cholecystography was repeated in eight of the nine later in convalescence and in each instance the gallbladder was visualized. Of the thirty-four patients whose gallbladders were visualized, seven were thirty years or more of age. Four of these patients had retention of bromsulfalein in amounts of 30 per cent or more, and in three of them the concentration of the radio-opaque dye was poor.

TABLE II

RELATIONSHIP OF AMOUNTS OF TOTAL SERUM BILIRUBIN AND PERCENTAGE OF RETENTION OF BROMSULFALEIN TO ROENTGENOGRAPHIC VISUALIZATION OF THE GALLBLADDER FOLLOWING THE INGESTION OF PRIODAX® IN PATIENTS WITH VIRAL HEPATITIS

Visualization by Cholecystography	Total Bilirubin mg./100 ml. Serum			% Bromsulfalein Retained†		
	2.0–4.99 (19)*	5.0–10.99 (17)*	11.0–24.0 (7)*	<15.0 (14)*	15.0–29.99 (18)*	30.0–66.0 (11)*
Good	13	13	0	8	17	1
Poor	4	3	1	4	1	3
None	2	1	6	2	0	7

* Number of patients.

† Forty-five minutes after the intravenous injection of 5 mg. dye/kg. body weight.

Of thirty-two jaundiced patients with retention of bromsulfalein below 30 per cent the gallbladder was visualized in twenty-nine (91 per cent), while only four out of eleven patients with 30 per cent retention or more had demonstrable shadows roentgenographically. In three of these the concentration of radio-opaque dye was poor. Thirty-six of the patients had a total serum bilirubin level below 11.0 mg. per cent, and thirty-three (92 per cent) had demonstrable gallbladders by oral cholecystography. In only one patient whose total

serum bilirubin measured 11.0 mg. per cent or more was the gallbladder visualized, and in this instance the dye was poorly concentrated. (Table II.)

The ten patients who concentrated the priodax® in the gallbladder sufficiently to be radio-opaque after a dose of twelve tablets, but not after a dose of six tablets, had total serum bilirubin determinations between 5.0 and 11.0 mg. per cent. Nine of these ten patients had retention of bromsulfalein between 15 and 30 per cent. Eight other patients concentrated the radio-opaque dye poorly but in these there was no correlation with degree of jaundice or retention of bromsulfalein since four had total serum bilirubin determinations between 2.29 mg. per cent and 5.0 mg. per cent, and retention of bromsulfalein below 15 per cent.

The ingestion of priodax® had no apparent untoward effect upon any of the forty-three patients with viral hepatitis, nor did it appear to alter their subsequent course of convalescence.

COMMENT

The data presented here indicate that when the total serum bilirubin is less than 11.0 mg. per cent and the retention of bromsulfalein less than 30 per cent, a large proportion of patients with viral hepatitis excrete priodax® and concentrate it in the gallbladder well enough to be visualized by roentgenogram. This may be of considerable clinical importance, particularly in patients of middle age or older age with relatively painless jaundice in whom the differential diagnosis between obstruction of the lower biliary tract and intrahepatic disease such as viral hepatitis may be difficult. Since the gallbladders of most of the patients whose total serum bilirubin measured 11.0 mg. per cent or greater and whose retention of bromsulfalein was above 30 per cent were not visualized, the use of cholecystography in this group is not warranted. It is probably advisable to give a double dose of radio-opaque dye orally (twelve priodax® tablets) to jaundiced patients whose total serum bilirubin is above 5.0 mg. per cent or to patients who have retention of bromsulfalein exceeding 15 per cent by the method used in this study.

SUMMARY

1. Cholecystography following the ingestion of priodax® was performed on forty-three jaundiced male patients in various phases of viral hepatitis.

2. The gallbladder was visualized in over 90 per cent of those whose serum bilirubin measured less than 11.0 mg. per cent and whose retention of bromsulfalein was less than 30 per cent. Most patients failed to concentrate the radio-opaque dye sufficiently to be visualized by roentgenogram if the amounts of total serum bilirubin and retention of bromsulfalein exceeded the above figures. In ten patients visualization of the gallbladder was obtained only by administering a double dose of radio-opaque dye.

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Clinical Course of Viral Hepatitis and the Effect of Exercise during Convalescence*

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THE variability of the clinical course of viral hepatitis has been described but the factors responsible for differences are not clearly defined.[1,2] The solution of this problem is made more difficult by the inability to determine if different outbreaks or cases of hepatitis are caused by the same virus or different viruses or variants of a single strain of virus. It is obvious, however, that certain factors which affect the host are also of importance in determining the course of disease. The influence of age has been repeatedly mentioned since the disease is usually mild in childhood and more severe in adult life, particularly after thirty years of age.[3–6] During recent years a highly fatal form of disease was observed in Denmark among older women beyond the menopause.[7] It has also been suggested that the physical condition of the patient before contracting hepatitis may be of importance and considerable variation in severity of disease was observed among American troops in World War II, with a more prolonged course and greater mortality rate among men who had been exposed to hardship and wounds in battle.[1,2,8] In addition, it has been emphasized that inadequate rest in bed and diet during the acute phase and early convalescence may prolong the disease.[1] The relative importance of these various factors is undetermined and it is likely that other things as yet undefined may alter the relationship between man and hepatitis virus.

Although the treatment of viral hepatitis is symptomatic and non-specific, emphasis has been given to the provision of prolonged rest in bed and excellent diet.[1,9–13] These principles of therapy were largely derived from the experience of Barker et al.[1,9] in the management of the disease in troops who had been debilitated by severe hardship in war. It has been suggested that this therapeutic regimen might be modified in younger adult patients who start their disease in relatively good physical condition.[14]

During the period from March, 1947, through October, 1949, 3,614 American soldiers and civilians with viral hepatitis were treated at the U. S. Army Hepatitis Center in Germany, providing an opportunity for observing various aspects of the course of disease. A tremendous number of results of different tests of hepatic function in these patients were accumulated and descriptions of various representative altera-

* From the 120th Station Hospital (Hepatitis Research Team), European Command, U. S. Army of Occupation in Germany, and the Jefferson Medical College of Philadelphia. This represents work done under the auspices of the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, Office of The Surgeon General, U. S. Army, Washington, D. C.

tions are recorded in other papers of this series.[15–19] The importance of bilirubinuria as an early diagnostic sign of epidemiologic value was suggested.[15] Increase in alkaline phosphatase and decrease in cholesterol esters in the blood during the acute phase of disease, with restitution of normal values in convalescence, were described.[16] Certain factors which influence the retention of bromsulfalein in the blood were studied and the results indicated that the ingestion of food before the test was associated with decreased retention of dye. Attempts to correlate the amounts of bilirubin and retention of bromsulfalein in the blood gave evidence of considerable dissociation in the capacity of the liver to excrete these two substances.[17] Comparison of the results of the cephalin-cholesterol flocculation, thymol turbidity and flocculation tests, and the colloidal red test indicated that the combined use of the first three tests gave the maximum assistance in the determination of hepatic parenchymal disease. The cephalin-cholesterol flocculation was more sensitive in the acute phase of disease but the thymol turbidity test remained positive longer in convalescence. Attention was also called to the considerable percentage of patients in whom negative results with all of these tests were obtained throughout the course of disease.[18] The capacity of patients with alteration of hepatic function to excrete radiopaque dye in amounts sufficient to be visualized by roentgenogram was studied and the value of this procedure in differential diagnosis of jaundice due to intra- or extrahepatic disease, particularly in older patients, was discussed.[19]

Attention has been called to the fact that the disease as seen during this time appeared to be milder than that occurring in Sicily, Italy or Germany during the years of World War II.[20] Whether this apparent modification was related in part to the better state of nutrition and lack of physical exhaustion at the time of onset or to a difference of strain of the causative organism is undetermined. It is the purpose of this paper to describe briefly the clinical course of disease in these patients and the effects of measured exercise on its course and duration. For the clinical analyses the pertinent data were assembled from the charts of several hundred patients during their hospitalization at the Hepatitis Center. The vast majority of these patients were soldiers who had received a good diet and were healthy and well nourished at the time of contracting hepatitis.

CLINICAL COURSE OF THE DISEASE

The course of disease in these patients was mild but otherwise not unusual with the exception of the type of onset. In contrast to other clinical descriptions of infectious hepatitis in troops in whom an acute onset with fever was generally noted[1,2] the onset of the disease described here was insidious in a large majority (76.4 per cent) of one group of 1,403 patients. Only 282 patients (20.1 per cent) had an acute onset and in forty-nine patients (3.5 per cent) the type of onset was not distinctly clear from the records. In those patients in whom the onset was acute the characteristic symptoms were anorexia, malaise and nausea, while only a small number had vomiting and diarrhea. Fever was uncommon and symptoms of coryza were rare. In the larger group of patients in whom the onset of disease was insidious the majority had no symptoms until dark urine was observed by the patient or scleral icterus by his comrades. Occasional patients in this group had mild malaise and intermittent anorexia for one to two weeks before the appearance of jaundice.

In the icteric phase fever was rarely recorded and the characteristic complaints of anorexia, nausea, vomiting, pain in the right upper quadrant and epigastric discomfort were present. In the mild cases these symptoms were slight and of short duration, while in the severely sick they persisted often for several weeks. Physical examination in this phase of disease revealed tenderness and enlargement of the liver in a large percentage of patients. Pruritus and posterior cervical adenopathy

were present in approximately one-third of the patients and splenomegaly with tenderness in the left upper quadrant occurred in only a small number of persons.

Course and Severity of Jaundice. Determinations of the amount of total serum bili-

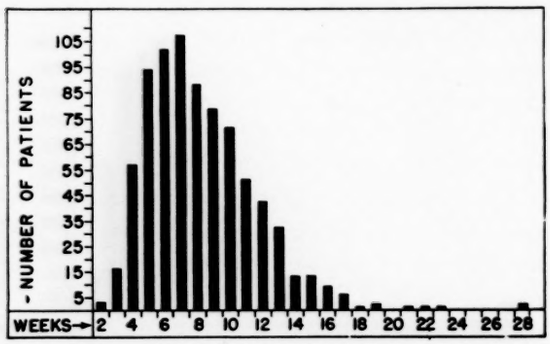

FIG. 1. Week of disease in which total serum bilirubin became normal (1.0 mg. per cent) in 790 patients with viral hepatitis.

rubin were made at admission to the hospital and usually at weekly intervals thereafter until normal amounts were found. The time in which the serum bilirubin first became normal varied between the second and the twenty-eighth week, averaging the eighth week, with greater duration of icterus in the more deeply jaundiced and older groups. (Figs. 1 and 2.)

Since a large majority of the patients were admitted to the Hepatitis Center during the third week of disease, it is possible that the maximum amount of total bilirubin may not have been measured in some; however, the records of 1,122 patients were analyzed in an effort to obtain information on the severity of jaundice as determined by the maximum amount of total bilirubin recorded. The results are tabulated in Table I. It is evident that a large percentage (61.9 per cent) of patients had mild disease as manifested by maximum measured amounts of total serum bilirubin of less than 5.0 mg. per cent. Although there was a wide range of time in which different patients attained the maximum measured amounts of total serum bilirubin, the average time of maximum icterus was in the third week, regardless of the severity of jaundice.

Course of Disease. The vast majority of the 3,614 patients observed at the Hepatitis Center made an uneventful recovery and were discharged from the hospital after an average period of sixty days. Twenty-four patients had moderately severe relapses

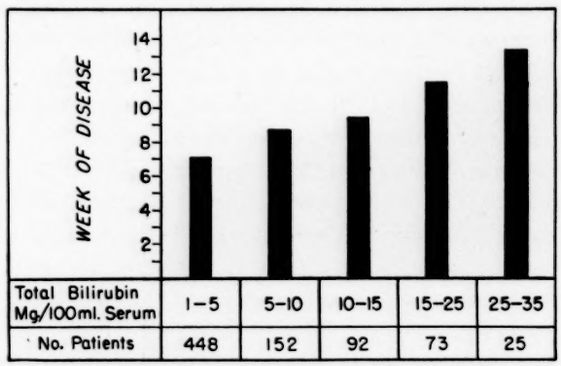

FIG. 2. Week of disease in which total serum bilirubin became normal (1.0 mg. per cent) in 790 patients with viral hepatitis, separated in groups according to maximum measured serum bilirubin attained during course of disease.

from which most of them recovered, chronic hepatitis developed in thirty-seven patients and two patients died. Seven other patients who were members of the Occupation forces in Germany or Austria during this time were too sick to be transported to the Center and died of fulminant hepatitis in other hospitals.

TABLE I
MAXIMUM MEASURED TOTAL SERUM BILIRUBIN DURING THE COURSE OF DISEASE OF 1,122 PATIENTS WITH VIRAL HEPATITIS

Maximum Total Bilirubin mg./100 ml. Serum	Patients	
	No.	%
<1.0	116	10.3
1.01– 5.0	579	51.6
5.01–10.0	183	16.3
10.01–15.0	116	10.3
15.01–25.0	98	8.7
25.01–51.0	30	2.7

EFFECT OF EXERCISE ON THE DURATION OF CONVALESCENCE

It was the practice at the Hepatitis Center to attempt to keep patients with

viral hepatitis in bed, with bathroom privileges, for at least three weeks after the onset of symptoms or until the patient was asymptomatic, without hepatic tenderness, and until the one minute serum bilirubin measured 1.0 mg. per cent or less. At this point in convalescence, patients were allowed out of bed with the privileges of walking to the mess hall, theater or recreation rooms. When the one-minute serum bilirubin measured less than 0.3 mg. per cent and the retention of bromsulfalein in the blood was normal,* and if the patient was asymptomatic, he was given a test of tolerance of exercise consisting of graduated calisthenics once daily for five days. During this period he was observed clinically and the determination of the amount of retention of bromsulfalein was repeated immediately following the fifth day of exercise. If the retention of bromsulfalein remained normal and there was no clinical evidence of relapse, the patient was discharged to duty.

Infractions against this regimen of therapy were common and it was not unusual to find men with considerable jaundice and other alterations of hepatic function indulging in a great deal more activity than prescribed. Because there was usually no apparent ill effect from such activity, an effort was made to evaluate the effect of measured exercise on the course of disease and the duration of convalescence of two groups of patients in various phases of disease. No conclusions can be drawn from this experiment regarding the effect of exercise on the occurrence of late sequelae or chronicity of disease.

TABLE II

AGE, PHASE OF DISEASE, DEGREE OF BILIRUBINEMIA AND CLINICAL OBSERVATIONS IN NINETY-EIGHT PATIENTS WITH VIRAL HEPATITIS

Group	No. of Patients									
	Age (yr.)		Weeks of Disease			Total Bilirubin mg./100 ml. Serum		Clinical Observations		
	<25	>25	2–3	4–7	>7	<3	>3	Hepatomegaly	Hepatic Tenderness	Symptoms
A (38)*	24	14	9	25	4	26	12	20	20	7
B (33)*	26	7	15	14	4	13	20	16	18	5
C (27)*	15	12	0	21	6	27	0	7	9	2

* Total number of patients in each group.

MATERIALS AND METHODS

Subjects. Ninety-eight patients in various phases of viral hepatitis, but all with diminishing bilirubinemia, were divided into three groups. None of these patients had fulfilled the criteria previously outlined as desirable before a trial of exercise was allowed. In Table II certain pertinent data are recorded concerning age, phase of disease, degree of jaundice and clinical observations in these subjects.

Group A was comprised of thirty-eight male patients ranging in age from nineteen to thirty-seven years in various periods of disease ranging from the second to the ninth week. The total serum bilirubin at the onset of the experiment ranged from 1.68 mg. per cent to 10.40 mg. per cent and the one-minute serum bilirubin from 0.68 mg. per cent to 4.18 mg. per cent. They were given graduated exercises morning and afternoon for two weeks, being kept at bed rest, with bathroom privileges only, except when taking the supervised exercises according to the following schedule, namely, the first day the exercises were calisthenics for ten minutes twice during the day, performed in bed, consisting of deep breathing and exercises involving motion

* 5 per cent or less forty-five minutes after the intravenous injection of 5 mg./kg.

of the extremities while remaining supine. More strenuous exercises were added each day and on the sixth day calisthenics in the standing posture were started. These were gradually increased in difficulty and duration until on the fourteenth day, after fifteen minutes of calisthenics in the morning, the patients were taken on a sixty-minute walk outdoors including fifteen minutes at double time.

TABLE III

RELATIONSHIP OF MEAN DURATION OF DISEASE TO AMOUNT OF BILIRUBINEMIA AT THE BEGINNING OF EXPERIMENT WITH EXERCISE

Group	Mean Duration of Disease (wk.)	Total Serum Bilirubin < 3 mg.		Total Serum Bilirubin > 3 mg.	
		No. of Patients	Mean Duration of Disease (wk.)	No. of Patients	Mean Duration of Disease (wk.)
A (38)*	9.7	26	9.1	12	11.1
B (33)*	9.6	13	10.0	20	9.5

* Total number of patients in each group.

Group B was comprised of thirty-three male patients ranging in age from nineteen to forty-one years in various periods of disease ranging from the second to the twelfth week of disease. They were used as control subjects and were kept in bed for fourteen days without exercise. The total serum bilirubin level of these patients at the start of the study ranged from 1.67 mg. per cent to 7.08 mg. per cent; the one-minute serum bilirubin was between 0.63 mg. per cent and 3.02 mg. per cent.

Group C was comprised of twenty-seven male patients ranging in age from nineteen to thirty-two years in periods of disease ranging from the fourth to the tenth week. The total serum bilirubin of these patients at the beginning of the study ranged from 1.10 mg. per cent to 1.98 mg. per cent and the one-minute serum bilirubin from 0.36 mg. per cent to 0.96 mg. per cent. Exercise was given to these patients according to the following schedule, namely, on the first day of the experiment the patients were given two periods of strenuous calisthenics, forty minutes in the morning and forty minutes in the afternoon, to the point of fatigue. Following the day of exercise the patients in this group were allowed unrestrained activity.

Clinical observations by the same physician were recorded daily with particular attention to the following, namely, anorexia, nausea and vomiting, pruritus, jaundice, size and tenderness of the liver, size and tenderness of the spleen, adenopathy and weight recorded twice weekly. The following tests of hepatic function were done twice weekly during the period of the experiment in Groups A and B, daily for three days on patients in Group C and at least weekly thereafter on all patients until discharge from the hospital, namely, total and one-minute serum bilirubin,[21] cephalin-cholesterol flocculation[22] and thymol turbidity.[23] Determinations of the excretion of two-hour and twenty-four hour urinary urobilinogen[24] and retention of bromsulfalein in the blood[25] were done at less frequent intervals. In Group C determinations of the excretion of twenty-four-hour urinary urobilinogen and twenty-four-hour total urinary coproporphyrin[26] were made on the day before exercise, the day of exercise, the day after exercise and twice weekly until discharge.

Except for the differences in activity allowed, the patients in all three groups were given the same treatment. The diet contained 4,000 to 4,500 calories, divided into 607 gm. of carbohydrate, 190 gm. of protein and at least 115 gm. of fat. In addition, each patient received three multivitamin tablets daily. Upon completion of the period of exercise or rest in bed the patients were allowed unrestrained activity and were discharged from the hospital when recovery was complete.

RESULTS

Duration of Disease. The mean duration of disease was the same in patients exercised and those kept in bed and the only constant relationship noted between exercise and prolongation of disease was that the use of exercise in patients whose total serum bilirubin measured more than 3.0 mg. per cent at the time of exercise appeared to

lengthen the period of convalescence. (Table III.) In agreement with the observations of others, the duration of disease appeared to be related to the age of the patient and the severity of jaundice. Thus, in patients of twenty-five years or over and in patients whose maximum one-minute serum bilirubin measured more than 5.0 mg. per cent, the course of disease was two weeks longer than in patients under twenty-five years and in patients whose maximum measured one-minute serum bilirubin was less than 5.0 mg. per cent. (Table IV.)

TABLE IV
RELATIONSHIP OF MAXIMUM MEASURED BILIRUBINEMIA TO MEAN DURATION OF DISEASE IN NINETY-EIGHT PATIENTS WITH VIRAL HEPATITIS

Group	Mean Duration of Disease (wk.)	
	Maximum 1′ Serum Bilirubin <5 mg.	Maximum 1′ Serum Bilirubin >5 mg.
A (38)*	8.5 (22)*	10.8 (16)*
B (33)*	8.9 (18)*	10.7 (15)*
C (27)*	9.2 (16)*	10.0 (11)*

* Number of patients.

However, there was no apparent prolongation of disease by exercise in the older age group or in those patients who had been most deeply jaundiced.

Clinical Observations. There was no significant difference in the incidence of complaints or physical signs of disease in the patients who exercised and the control subjects. Soreness of muscles was a frequent symptom among patients in Group C immediately following the one day of abrupt, rather strenuous exercise. The most common complaints in order of frequency were epigastric pain, anorexia and discomfort in the right upper quadrant. Nausea and vomiting were rare. In occasional patients in both groups who exercised and in the control group, also, slight apparent enlargement and/or transient tenderness of the liver to palpation developed. Those patients whose livers became tender during exercise and in whom exercise was continued had somewhat longer courses of disease than other members of their groups, while in the control group the occurrence of hepatic tenderness was not associated with any apparent increase in duration of disease.

Laboratory Observations. The alterations in the results of the various determinations of hepatic function were slight and revealed no appreciable differences which could be interpreted as due to the regimen imposed on the different groups of patients, with the exception of certain increases in excretion of urinary urobilinogen and coproporphyrin in Group C. The alterations in the various tests are tabulated in Table V. The fact that more patients on a regimen of rest in bed had slightly increased positivity of cephalin-cholesterol flocculation tests and retention of bromsulfalein dur-

TABLE V
ALTERATIONS IN VARIOUS TESTS OF HEPATIC FUNCTION IN NINETY-EIGHT PATIENTS WITH VIRAL HEPATITIS AFTER EXPERIMENTS WITH EXERCISE

Group	No. of Patients with Increased Abnormality in Tests of Hepatic Function						
	Serum Bilirubin	Cephalin-Cholesterol Flocculation	Thymol Turbidity >2 units	Bromsulfalein Retention	2-hr. Urine Urobilinogen	24-hr. Urine Urobilinogen	Total Coproporphyrin
A (38)*	1	5	1	3	11	1	..
B (33)*	1	12	1	7	8	1	..
C (27)*	2	0	3	..	..	13	18

* Total number of patients in each group.

ing the experiment is probably of no significance.

It is of interest that thirteen of twenty-seven patients in Group C had urobilinogenuria increased to an abnormal level within a week following exercise. In two patients this was accompanied by a slight increase in bilirubinemia; in two others hepatic tenderness became evident and in one other patient mild, transitory pain in the right upper quadrant occurred. However, the mean duration of illness of these thirteen patients with increased urobilinogenuria was shorter than that of all patients of the group. Eighteen patients in Group C had an increase in excretion of total coproporphyrins within a few days after exercise but there was no significant difference in duration of disease between those who demonstrated increased urinary coproporphyrin excretion following exercise and those who did not.

COMMENT

The insidious onset of disease without fever in a large percentage of the patients described here constitutes a major difference from previous clinical descriptions of infectious hepatitis.[1,2,5] The question has been raised as to whether it may not indicate an unusual amount of artificial transmission of disease by means of parenteral inoculation since homologous serum hepatitis characteristically begins insidiously and without fever.[27] In the present state of knowledge it is impossible to determine this point, although it is believed that ample opportunity existed for this situation to develop.[28]

The mildness of viral hepatitis in American Occupation forces in Germany during the past three years is attested by the large percentage of patients (61.9 per cent) with slight jaundice, the low rate of mortality and the relatively rare occurrence of relapse or chronic hepatitis. Although it is possible that undetected examples of these latter complications exist, it would be unlikely for their number to be great without attracting attention. In support of this are the results of follow-up examination of two groups (approximately 100 each) of patients six months to one year after recovery from hepatitis in which no significant evidence of residual hepatic disease was found.[20,29]

Although a large percentage of patients had mild jaundice, the average time for the whole group to attain a normal amount of total serum bilirubin was in the eighth week of disease. This did not indicate the exact duration of the icteric phase since these determinations were made only at weekly intervals and, in addition, no account was taken of the duration of the pre-icteric phase in those patients in whom it was present. The average period of hospitalization (sixty days) reflects in part a conservative regimen of therapy and, in addition, is related to the particular conditions of military life in which a patient returning to duty must be ready to undertake any activity.

The data presented in the second part of this study support the recent clinical observations that activity in excess of that usually prescribed for patients convalescing from viral hepatitis apparently may not be injurious or prolong the course of disease when certain criteria are observed. The amount of jaundice present at the time planned exercises were started appeared to be an important indication of the capacity of the patient to tolerate increased activity. Although the numbers of patients in each group studied are small, the results suggest that convalescence may be prolonged if exercise is allowed when the total serum bilirubin measures more than 3.0 mg per cent. When the serum bilirubin measured less than this at the beginning of exercise, the duration of convalescence was apparently not prolonged by exercise as compared with rest in bed, regardless of the age of the patient, severity of disease as measured by the maximum amount of serum bilirubin previously attained or the period of disease in which exercise was given.

There were no significant differences in the occurrence of symptoms, signs or increased impairment of hepatic function, as

determined by the tests performed, in the two groups of patients who exercised and the group kept in bed. In retrospect, the most important indications of an untoward effect of exercise were the occurrence of anorexia, hepatic tenderness or an increase in retention of bromsulfalein. However, patients in all three groups had one or more of these alterations without sustaining any prolongation of disease with the exception that those patients whose livers became tender during exercise and who continued to exercise had somewhat longer courses of disease than other members of their group. In contrast, the occurrence of hepatic tenderness in patients who remained in bed was not associated with any apparent increase in duration of disease. Five patients (three exercised and two remained in bed) had convalescence prolonged beyond 100 days but there was little to indicate this protracted convalescence during the course of the experiment. That all but one of these men were over twenty-five years of age and had maximum one-minute serum bilirubins greater than 5.0 mg. per cent is in accord, however, with the general experience that convalescence is frequently prolonged in older or more deeply jaundiced patients.

The fact that a somewhat less conservative regimen of therapy for previously healthy young adults with viral hepatitis is not associated with any apparent ill effects is in accord with the experience in treatment of the disease in children to whom less stringent rules of therapy have usually been applied. Under these conditions it would appear valid to modify the regimen which was derived largely from experience with the disease in troops exhausted by hardship and wounds. The ease and convenience of performance of the test of tolerance for hard exercise in one day as performed by patients in Group C suggests a further trial of its value as a means of determining return to activity.

SUMMARY

1. Data from 3,614 cases of viral hepatitis in American Occupation forces in Germany occurring between March, 1947, and October, 1949, were assembled and analyzed.

2. The course of disease was mild but similar to previous clinical descriptions with the exception of the onset which was insidious and afebrile in a large majority of patients.

3. Since a great many of these patients had much more activity at an earlier phase of convalescence than is usually prescribed without apparent ill effect, an attempt was made to determine the effect of measured exercise on the duration of disease.

4. Thirty-eight patients in various phases of viral hepatitis were given a two-week period of graduated exercise and thirty-three similar patients were kept in bed as controls. Twenty-seven other patients did strenuous calisthenics for one day.

5. The mean duration of disease was two weeks longer in patients whose total serum bilirubin measured 3.0 mg. per cent or more at the onset of exercise. The most important indications of an untoward effect of exercise were anorexia, hepatic tenderness or an increase in retention of bromsulfalein.

6. When the total serum bilirubin measured less than 3.0 mg. per cent at the beginning of exercise, the duration of convalescence was apparently not prolonged by exercise as compared with rest in bed, regardless of the age of the patient, severity of disease as measured by the maximum amount of serum bilirubin previously attained or the period of disease in which exercise was given.

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Clinical Studies

Myocardial Infarction without Anticoagulant Therapy*

Deaths, Emboli and an Analysis of Factors Influencing Mortality

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THIS paper presents an analysis of 414 consecutive bonafide cases of myocardial infarction not treated with anticoagulants seen at the University Hospital† between 1935 to 1948. These findings suggest that the statistical evidence of the effectiveness of anticoagulant therapy thus far reported may be unintentionally misleading in some respects.‡ We find that age, sex, pre-existing hypertension, the presence of angina and previous infarction materially influence the statistical probability of death from myocardial infarction. It is our opinion that unless such factors are weighed accordingly in both treated and untreated subjects, evaluation of important advances in therapy is unsatisfactory. The present series, together with those from the literature so analyzed, we believe provide a suitable outline of at least some of the factors to be considered in setting up a control and treated group for the subsequent evaluation of the usefulness of anticoagulants and other forms of therapy in this disease.

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‡ Study begun independently in April, 1946, at Cincinnati General Hospital and has continued since February, 1947, in collaboration with the Committee on the Use of Anticoagulants in the Treatment of Coronary Thrombosis with Myocardial Infarction of the American Heart Association.[3] Although an attempt has been made by alternating days or cases to have an adequate control group, it is apparent from the following data that this alone is insufficient to provide an adequate control series.

METHODS AND MATERIAL

The present series includes 414 consecutive cases of myocardial infarction of which 334 occurred in men and 80 in women. In each a typical history was substantiated by laboratory and electrocardiographic findings except in eleven patients who died shortly after admission. Anticoagulant therapy was given to three patients, in each instance only after embolization had occurred.

Thromboembolism in survivors and in those fatal cases not autopsied was diagnosed in the presence of characteristic signs and symptoms (i.e., peripheral thrombosis and/or the appearance of emboli to lungs, brain, etc.). It is our impression that major thromboembolic phenomena were not missed although it is likely that minor or deep-seated episodes occurred among some survivors. In any event, these latter if present were not of sufficient significance to impede recovery or prolong hospitalization. Among those on whom necropsy was performed the number of thromboembolic phenomena is recorded.

A summary of the data relating to age, sex, deaths, pre-existing hypertension, angina and previous infarctions among our patients is presented in Table I. Data from the literature on age distribution and death are presented in Table II.

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RESULTS

The age distribution of our cases was studied together with that of those cases previously reported for the purpose of determining if there was any basic dissimilarity in our series and a composite of previously reported series. If a significant difference in age distribution is found, all other factors to be studied become biased by it.

The age distribution among 3,396 pa-

TABLE I

A SUMMARY OF DATA FROM OUR SERIES

Years	30–39				40–49				50–59				60–69				70				Total No. of Cases
Infarction	1st	2nd	3rd	Total	1st	2nd	3rd	Total	1st	2nd	3rd	Total	1st	2nd	3rd	Total	1st	2nd	3rd	Total	
Age Distribution																					
Male	13	3	2	18	72	11	2	85	144	12	..	126	72	12	1	85	15	5	..	20	334
Female	1	..	..	1	3	..	..	3	33	2	..	35	22	5	..	27	13	..	1	14	80
Total cases	..	..	..	19	..	..	..	88	..	..	..	161	..	..	..	112	..	..	..	34	414
Deaths																					
Male	1	..	..	1	1	1	1	3	9	6	..	15	15	6	1	22	3	2	..	5	46
Female	..	..	..	0	..	..	..	0	6	..	..	6	6	2	..	8	4	..	..	4	18
Total	..	..	..	1	..	..	..	3	..	..	..	21	..	..	..	30	..	..	..	9	64
Hypertension																					
Male	2	..	..	2	21	3	1	25	38	4	..	42	32	4	..	36	6	1	..	7	112
Female	..	..	..	0	1	..	..	1	18	1	..	19	8	4	..	12	8	..	1	9	41
Total	..	..	..	2	..	..	..	26	..	..	..	61	..	..	..	48	..	..	..	16	153
Deaths	..	..	..	0	..	..	..	1	..	..	..	15	..	..	..	14	..	..	..	5	35
Angina																					
Male	3	..	..	3	20	5	..	25	36	9	..	45	26	7	1	34	4	2	..	6	113
Female	..	..	..	0	2	..	..	2	10	1	..	11	9	3	..	12	..	..	..	0	25
Total	..	..	..	3	..	..	..	27	..	..	..	56	..	..	..	46	..	..	..	6	138
Deaths	..	..	..	1	..	..	..	2	..	..	..	9	..	..	..	14	..	..	..	2	28
Angina and Hypertension																					
Male	..	..	..	1	..	..	..	10	..	..	..	17	..	..	..	16	..	..	..	3	47
Female	..	..	..	0	..	..	..	1	..	..	..	6	..	..	..	9	..	..	..	0	16
Total	..	..	..	1	..	..	..	11	..	..	..	23	..	..	..	25	..	..	..	3	63
Deaths	..	..	..	0	..	..	..	1	..	..	..	5	..	..	..	6	..	..	..	2	14

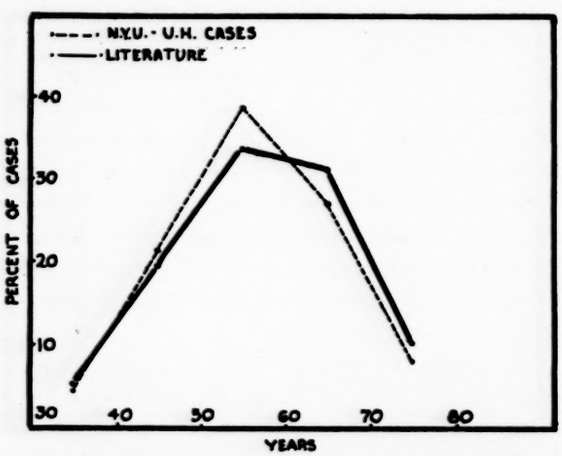

FIG. 1. The broken line represents the age distribution curve of the 414 men and women in the New York University — University Hospital series. The solid line represents the age distribution curve of 2,982 men and women from the literature together with our group of 414 patients with myocardial infarction.

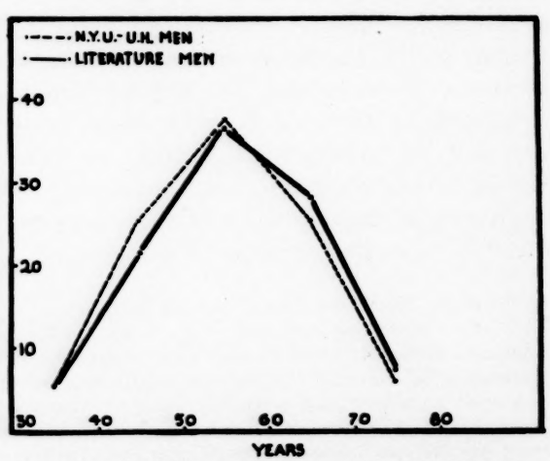

FIG. 2. The broken line represents the age distribution of the 334 men in the New York University — University Hospital series. The solid line represents the age distribution curve of 1,509 men with myocardial infarction in the literature together with our 334 men.

tients[4–18] is shown graphically in Figure 1. Figures 2 and 3 indicate the relative number of males and females in the various age groups.[6,7,9,14,16,17] The difference in the percentage of deaths at various ages in myocardial infarction is shown in Figure 4 in

TABLE II
A SUMMARY OF DATA FROM THE LITERATURE

Author	Years					
	30–39	40–49	50–59	60–69	70+	Total
Age Distribution Total						
Motoshaw[18]	20	38	15	8	1	82
Silberberg[17]	18	55	105	113	27	318
Jervell[16]	1	7	21	24	12	65
Rathe[15]	4	38	80	101	51	274
Mullins[21]	27	92	135	111	32	397
Bancher[12]	5	9	15	23	3	55
Harrington and Wright[13]	4	21	64	36	23	148
Levine[11]	3	29	44	55	14	145
Tredway[10]	0	16	22	28	9	75
Willius[9]	6	63	148	118	35	370
Parkinson and Bedford[8]	2	20	33	35	10	100
Master, Jaffe and Dack[7]	21	62	86	71	27	267
Master, Dack and Jaffe[6]	39	105	178	141	37	500
Coombs[5]	3	7	17	45	14	86
Ney and Barnes[4]	2	19	29	33	17	100
Total	155	581	992	942	312	2982
Age Distribution Male						
Master, Jaffe and Dack[7]	15	46	70	53	19	203
Master, Dack and Jaffe[6]	33	85	139	103	27	387
Willius[9]	6	60	135	99	24	324
Silberberg[17]	13	47	88	85	19	252
Jervell[16]	0	5	16	15	9	45
Mullins[14]	17	73	104	84	20	298
Total	84	316	552	439	118	1509
Age Distribution Female						
Master, Jaffe and Dack[7]	6	16	16	18	8	64
Master, Dack and Jaffe[6]	6	20	39	38	10	113
Willius[9]	0	3	13	19	11	46
Silberberg[17]	5	8	17	28	8	66
Jervell[16]	1	2	5	9	3	20
Mullins[14]	10	19	31	27	12	99
Total	28	68	121	139	52	408
Deaths Total						
Mullins[14]	0	4	10	13	7	34
Tredway[10]	0	4	4	10	3	21
Rathe[15]	1	8	17	21	8	55
Willius[9]	0	5	31	21	13	70
Master, Jaffe and Dack[7]	2	8	13	15	6	44
Master, Dack and Jaffe[6]	6	22	43	60	13	144
Total	9	51	118	140	50	368

our series combined with those cases in the literature in which such data were available.[6,7,9,10,14,15] The percentage of deaths at various ages according to sex in our series is shown in Figure 5. The only report found which provides similar data is that of Willius.[9]

Hypertension. In our series 153 (36.7 per cent) had known hypertension. All of these either presented a diastolic blood pressure of 100 mm. Hg or above while under our care or a reliable history of hypertension was available. This, of course, excludes those patients with hypertension previous to infarction in whom the pressure fell to

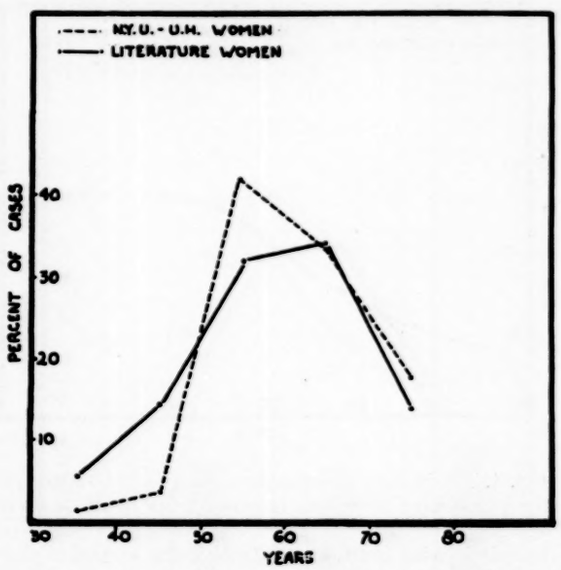

FIG. 3. The broken line represents the age distribution curve of the eighty women in The New York University — University Hospital series. The solid line represents the age distribution curve of 408 women with myocardial infarction together with our eighty women.

normal levels and stayed there while under our observation.

Of 4,035 cases reported in previous studies[4,6,7,9,15,18–30] 46 per cent were reported to have hypertension. The criteria for hypertension varied from 140/90 to 160/110 mm. Hg.

From our University Hospital group and those reported previously in which sex distribution was noted[6,11,18,25,26] a total of 1,745 cases (1,334 men and 420 women), hypertension was found to occur in 37.2 per cent of the men and in 63 per cent of the women.

The discrepancy between the frequency of hypertension in our group and in the literature is partly explained by the variance in standards for hypertension used and partly by the difference in sex ratio. In the previously reported series the ratio of

women to men was 1 to 3.2 and in our group 1 to 4.2.

The hazard to the patient with pre-existing hypertension in the event of myocardial infarction is shown graphically in Figure 6. Of our 153 hypertensives 22.8 per

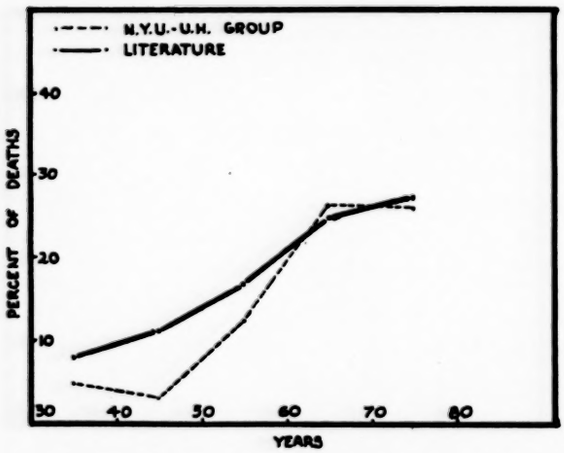

FIG. 4. The broken line connects points which represent the percentage of deaths (mortality per cent) in each age group for the New York University — University Hospital series and the solid line for the literature plus our group.

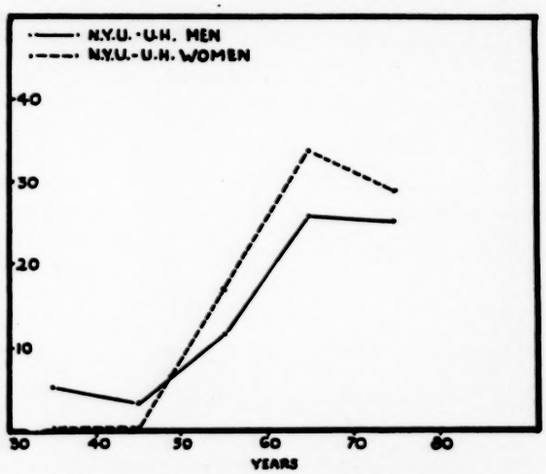

FIG. 5. The broken line connects points which represent the percentage of deaths (mortality per cent) in each age group for our women and the solid line for our men (New York University — University Hospital series).

cent died and of our 261 normotensives 11.9 per cent died. Of the 222 normotensive males 11.7 per cent died; of 112 hypertensive men 17.8 per cent died; 31.7 per cent of forty-one hypertensive women died and only 12.8 per cent of thirty-nine normotensive women died. These differences cannot be attributed to age distribution of the normotensive and hypertensive groups because as shown in Figure 7 the mortality rate in each of the older age groups is distinctly higher among hypertensives than among normotensives.

These findings are in agreement with those of Rosenbaum and Levine[30] who used a similar standard for hypertension. They found a mortality rate of 36 per cent for patients with hypertension and a mortality rate of 24 per cent for patients without hypertension. Master, Dack and Jaffe[6] and Mintz and Katz,[26] using a diastolic pressure of 90 mm. Hg as a standard, found no significant difference in the mortality rate with or without hypertension. Baker and Willius[31] and Levine[11] report no significant differences in mortality rates with or without hypertension. The majority of authors have not analyzed this factor in their reports of mortality in myocardial infarction. Pre-existing hypertension, therefore, in both males and females in our experience is of prognostic importance in the outcome of myocardial infarction.

Angina. Of our 414 patients 33.3 per cent gave a convincing history of angina which had existed at least one month before the myocardial infarction. Of 3,315 cases reported in the literature[4, 9, 12, 15, 19, 21, 23, 24, 26, 29, 30, 32–35] 44.0 per cent had angina prior to the myocardial infarction. However, many of these included angina of less than a month's duration. The incidence of angina in the 334 men in our series was 33.8 per cent and in the 80 women was 31.3 per cent. This similarity in incidence agrees with the findings of Willius.[9]

Of the 138 men and women with angina of one month or more prior to infarction 20.3 per cent died. Of these sixty-two patients had both hypertension and angina but the percentage of deaths was not significantly altered (22.6 per cent). Similarly, death occurred in 23.1 per cent of ninety-one patients who had previous hypertension

but no previous angina. There were seventy-six patients who had previous angina but no hypertension. Of these 18.4 per cent died of their infarction. It is of some interest that of 185 patients who had neither previous angina nor hypertension 8.1 per cent died. (Table III.) This is not the effect of age alone because, as shown graphically in Figure 8, the mortality rate in each age group is definitely higher among those patients with angina or hypertension than it is among those without either angina or hypertension.

TABLE III
EFFECT OF ANGINA AND HYPERTENSION ON OUR MORTALITY RATE

No. Patients	Per cent of Deaths
62 with hypertension and angina	22.6
91 with hypertension alone	23.1
76 with angina alone	18.4
229 total	21.4
185 with neither hypertension nor angina	8.1

In the large series of Mintz and Katz[26] and the small series of Tredway[10] similar findings are reported in regard to angina. Woods and Barnes[34] found no appreciable difference in the two groups and Rosenbaum and Levine[30] found the mortality rate to be lower for those patients who had angina prior to the infarction.

Diabetes. Thirty-two (7.9 per cent) of our entire group showed evidence of diabetes. This represented 5.1 per cent of the men and 18.9 per cent of the women. In ten patients who died shortly after admission no information was available on which to base this diagnosis. Five of the known diabetics died (15.6 per cent).

Among previously reported groups[4,6,10–12,18–20,24,26,27,29,31] 265 (11.1 per cent) of 2,389 cases were diabetics. Of 890 men 74 (8.3 per cent) and of 327 women 101 (30.9 per cent) had diabetes.[6,11,26] Significant differences in mortality rate with or without diabetes were not found in our group or in the previously reported groups.

Influence of Multiple Infarctions on the Mortality Rates. In our University Hospital group of 358 men and women who had their first myocardial infarction the percentage of deaths was 12.6 per cent. This falls between previously reported rates which were lower[4,7,14] and those which were higher.[6,15,19,25,27,29,30,34,36] The over-all percentage of deaths for 2,357 cases so reported in the literature[4,6,7,14,15,27,29,30,34,36] and including our series was 18.3 per cent.

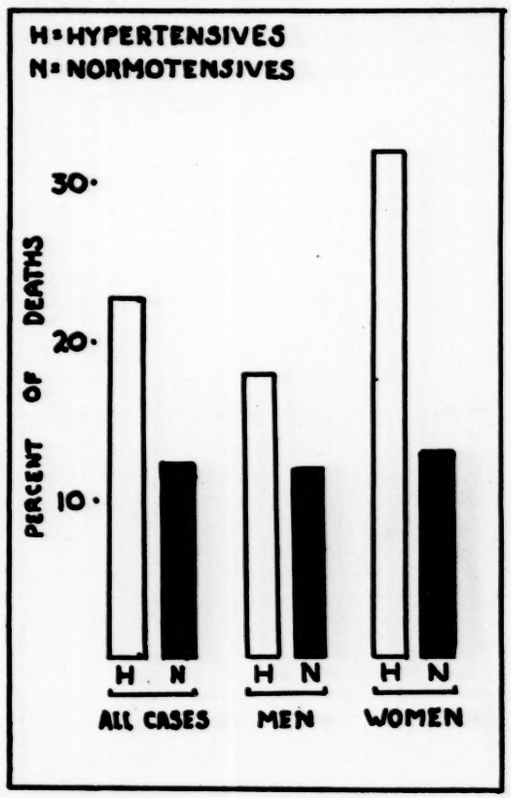

FIG. 6. The solid blocks represent the percentage of deaths among normotensives for men, women and all cases. The open blocks represent percentage of deaths among hypertensives for men, women and all cases in the New York University — University Hospital series.

In our group of fifty patients who had their second myocardial infarction the percentage of deaths was 34 per cent. Of the 483 cases previously reported plus our 50 cases, a total of 533 cases, the percentage of deaths was 30 per cent.

In our series only six patients had a third infarction to our knowledge. Of these, two succumbed. Eighty-three cases were previously reported[6,7,28,29] which with ours

gives a total of 89 cases; 35.9 per cent of this group died.

It follows from these data that the chances of survival decrease with each additional major infarction. It is also probable from a study of autopsy series such as that of Blumgart, Schlesinger and Davis[48] that a good number of clinical cases carried as first infarctions have had one or more previous infarctions. Edmondson and Hoxie[37] found among 865 autopsies of patients with unhealed infarcts that 55.7 per cent had old scars. Lisa and Ring[38] found in thirty-two cases of recent infarction twenty-three had evidence of an old infarction. That this situation exists also in younger age groups is evidenced by French and Dock,[69] 59 per cent of whose eighty cases under forty years of age had a previous infarction. Poe[40] reviewing infarctions among the military noted that evidence of old myocardial damage was frequent.

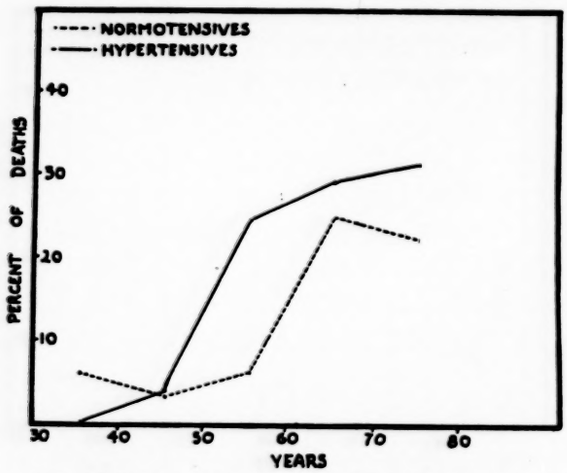

FIG. 7. The broken line connects points which represent the percentage of deaths (mortality per cent) in each age group among those patients in the New York University — University Hospital series who were normotensives and the solid line shows the percentage of deaths in age groups among the hypertensives.

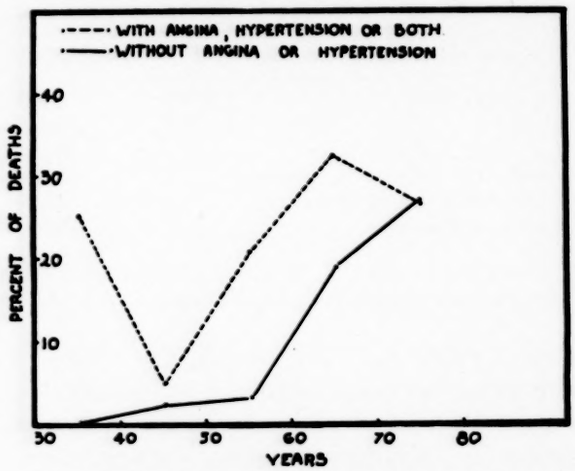

FIG. 8. The broken line connects points which represent the percentage of deaths (mortality per cent) in each age group among those patients in the New York University — University Hospital series who had angina, hypertension or both and the solid line shows the percentage of deaths in each age group among our patients who had neither angina nor hypertension.

Thromboembolic Phenomena. In our University Hospital group of 414 patients major thromboembolic phenomena occurred in ten of the survivors. Of the thirty-four patients who died and who did not come to necropsy only three showed clinical evidence of a major thromboembolic phenomenon. Of the thirty patients who died and in whom necropsy was performed five patients showed evidence of embolic phenomena, ten had mural thrombi and in three additional patients thrombotic phenomena were found elsewhere. In three of these patients the necropsy was limited to the heart; 6.5 per cent of our 414 patients had one or more thromboembolic phenomenon.

The autopsy findings are shown in Table IV which is included for the purpose of pointing out the frequency of cardiac hypertrophy, congestive failure and pneumonitis. It is notable that of the ten patients who had a mural thrombus at necropsy it was found in nine to be in the left ventricle and in only one of these cases were there associated embolic phenomena which could not be considered the cause of death. In one case in which a mural thrombus was found in both ventricles the necropsy unfortunately was limited to the heart. In Table V is shown our experience and in Table VI that of other authors with thromboembolic phenomena in their series of myocardial infarctions.

Localization of Infarctions. Of our 414 patients 223 had anterior myocardial infarction (53.9 per cent). The mortality rate for anterior myocardial infarctions was 13.5 per cent; 151 patients had posterior myocardial infarctions (36.5 per cent) with a mortality rate of 9.3 per cent; twenty-nine patients had infarctions (7.0 per cent) which could not be accurately localized due mainly to bundle branch block and in this group the mortality rate was 31.0 per cent. The eleven patients (2.7 per cent) in whom no electrocardiogram was available died shortly after admission.

Percentage of Deaths for All Cases. In the University Hospital group of 414 cases the percentage of deaths was 15.5 per cent including deaths in men and women in all categories, first, second and third infarctions.

In the 4,108 cases in the literature[4,6–13,23,25,26,29,34,35,42–45] and our 414 cases the combined mortality rate was 23.5 per cent, which includes deaths in all categories,

TABLE IV

Case Number	Mural Thrombus	Lungs	Kidney	Colon	Thrombosis	Hypertrophy	Dilatation	Pericarditis	Chronic Passive Congestion	Failure	Other Disease
1	L.V.	..	+	..	.....	..	..	..	+	.....	Interstitial pneumonia
2	.....	..	..	..	.....	..	..	..	+	Hydrothorax	Bronchiectasis, cancer of lung, rheumatic heart disease
3	.....	..	..	..	.....	..	..	..	+	Hydrothorax, ascites	
4	L.V.	..	..	..	.....	..	..	..	+	.....	Lobular pneumonia, cancer of stomach with metastasis
5	.....	..	..	..	.....	+	+	+	..	Hydrothorax	Patent foramen ovale
6	L.V.	..	..	..	.....	+	+	+	+	.....	Atelectasis
7	.....	..	..	..	.....	+	+				
8	.....	..	..	..	.....	..	+	..	+	.....	Cyst of kidney, calculus left kidney, chronic pyelitis, left
9	.....	+	..	..	Pulmonary artery	+	+	..	+	Hydrothorax	
10	.....	..	..	..	.....	+	..	..	+	.....	Cysts of kidney
11	.....	+	..	+	.....	+	..	..	+		
12	L.V.	+	..	..	.....	..	..	..	+	Hydrothorax	Lobular pneumonia
13	L.V.	+	..	..	Pelvic veins	+	+	+	+	Hydrothorax	Lobular pneumonia, rheumatic heart disease, patent foramen ovale
14	L.V.	..	..	..	.....	+	+	..	+		
15	.....	..	..	..	.....	+	..	..	..	Congestion of lungs, acute	Cancer of prostate
16	.....	..	..	..	.....	..	..	..	+	Hydrothorax	Cholelithiasis
17	.....	..	..	..	.....	..	..	..	+	Hydrothorax	Lobar pneumonia
18	.....	..	..	..	.....	+	..	..	..	.....	Rheumatic heart disease, hydronephrosis, left, suppurative pyelonephritis, right
19	.....	..	..	..	.....	+	..	..	+	.....	Congenital cysts kidney, nephrolithiasis, left
20	.....	+	..	..	.....	..	..	..	+	Hydrothorax	
21	.....	..	..	..	Pelvic veins	+	..	..	+		
22	.....	..	..	..	.....	..	..	..	+	Edema lungs	Chronic pyelonephritis
23	L.V.	..	..	..	Abdominal aorta, mural; pelvic veins	..	..	+	+		
24	.....	..	..	..	.....	+	+	..	+	Hydrothorax, hydropericardium, edema lower extremities	Lobular pneumonia, polycystic kidney, left
25	.....	..	..	..	.....	+	+	..	+	Hydrothorax	Cholesterolosis of gall bladder
*26	.....	..	..	..	.....	+	..	..	..	.....	Cholelithiasis
*27	L.V. & R.V.	..	..	..	.....	+	+				
28	L.V.	..	..	..	.....	..	..	..	+	Hydrothorax, ascites, edema lower extremities	Acute ulcer, stomach, lobular pneumonia, perforation right ventricular wall with hemopericardium
29	L.V.	..	..	..	.....	..	..	+	+	Hydrothorax	Atelectasis
*30	.....	..	..	..	.....	+	+				

* Necropsy limited to the heart.

first, second and third, irrespective of sex. It should, however, be noted that these figures include such early series as those of Parkinson and Bedford[8] published in 1928, Levine[11] published in 1929 and Coombs[35] published in 1932. Coombs noted at that time that with increasing accuracy in early diagnosis the prognosis was improving.

TABLE V
THROMBOEMBOLIC PHENOMENA—OUR SERIES

Emboli	Autopsy	No Autopsy	Survivors
Pulmonary	5	2	1
Cerebral	0	1	5
Kidney	1	0	0
Extremities	0	0	5
Colon	1	0	0
Thrombosis	5	0	1
Mural thrombus	10	0	0

The combined mortality rate[6,7,9,26,34,45] for 1,651 men in the literature together with our 334 men is 20.7 per cent and for 486 women plus our 80 women is 30.4 per cent. both treated and untreated patients must be classified as to sex, age groups and previous status in order that an unbiased comparison may be made.

The use of average ages is misleading since significantly different age-distribution curves may give identical figures for average age. A difference in mortality rate may result as a reflection of the masked age distribution rather than as the result of the variable in question. A striking example of marked dissimilarity in age-distribution curves with identical average ages is seen in the control and treated groups in the series of Glueck, Strauss, Pearson and McGuire.[3] (Fig. 9.) This example indicates the direction of bias that can lead to unintentionally exaggerated conclusions although in this instance statistically the bias is not very great.

Furthermore the mortality rate from myocardial infarction is about 50 per cent higher in women than in men. It follows that sex distribution will influence the mortality rate. In the series of Glueck et al.[3] the ratio of women to men in the treated group is 1 to 7.33 whereas in the control group it is 1 to 2.57; and in the series of Peters et al.[1] it is 1 to 4.55 in the treated group and 1 to 2.33 in the control group.

That the mortality rate rises with the number of infarctions is well known. Glueck

TABLE VI
DATA FROM LITERATURE ON EMBOLIC PHENOMENA IN MYOCARDIAL INFARCTION

Author	Per cent of Patients with Emboli	No. of Cases	Lung	Brain	Kidney	Mesentery	Spleen	Retina	Limb
Howard[19]	10.3	165	9	3	2	..	..	..	3
Connor and Holt[29]	14.6	267	21	14	8	3	..	1	2
Parkinson and Bedford[8]	8.0	100	3	3	1	..	1		
Blumer[41]	15.4	175	16	9	1	9			
Rosenbaum and Levine[30]	10.5	208	9	9	3	..	6	..	1
Woods and Barnes[34]	6.2	128	6	..	..	..	..	..	2
Ney and Barnes[4]	26.0	100	14	8	..	..	..	..	4
Mintz and Katz[26]	9.9	572	28	11	3	1	2	..	6
Harrington and Wright[12]	7.0	148	9	1	..	..	..	..	1
Scarlett[27]	8.0	64							
Our series	4.6	414	8	6	1	..	..	..	5
Total with our series	10.8	2,341							

COMMENT

It is apparent from the data presented in this report that the possibility of death from myocardial infarction is materially affected by sex, age and the presence or absence of previous infarction. Thus it follows that in the evaluation of therapeutic procedures

et al.[3] report that 16 per cent of their treated group and 32 per cent of their control group had a previous myocardial infarction. The number of patients in the control group who had had a previous infarction was not recorded by Greisman et al.[2] nor by Peters et al.[1]

From the analysis of our series it is apparent that hypertension has an adverse effect on the outcome of myocardial infarction. It is recommended that in an evaluation of the effect of therapy the incidence of hypertension in the treated and control group be taken into account and the percentage of deaths be defined for hypertensives and normotensives in the control and treated group. The frequency of hypertension is not mentioned by Wright and Marple,[46,47] Peters et al.[1] and Greisman et al.[2] in the data relating to their treated and control groups. Glueck et al.[3] note that 60 per cent of their treated group and 44 per cent of their control group had hypertension.

From our data it is apparent that angina and other evidence of cardiac embarrassment, as would be expected, have an unfavorable influence on the mortality rate. Peters et al.[1] found it necessary to use digitalis in 16 per cent of the treated group and in 28.3 per cent of the controls. Glueck et al.[3] used digitalis in 12 per cent of their treated group and in 40 per cent of their control group. It is likely that this is a further indication of the inequality of their control observations. Greisman et al.[2] have not given sufficient information about their control group to allow comparison of the control and treated group beyond age distribution and sex which match closely in this series.

Major thromboembolic phenomena, when they occur, influence the recovery of the patient unfavorably and result in death, disability or at least in prolongation of the hospital stay. However, in our series we have found thromboembolic phenomena to have little effect on the mortality rate. In one case only could death be directly ascribed to thromboembolism. That case is No. 11, Table IV, in which an embolus lodged in the pulmonary artery. Many authors (Table VI) point out that thromboembolism occurring among patients in their series who died was not the cause of death. For example, Conner and Holt[29] found that fifteen patients who had one or more embolic phenomena died but not necessarily from the immediate effects of the embolic attack.

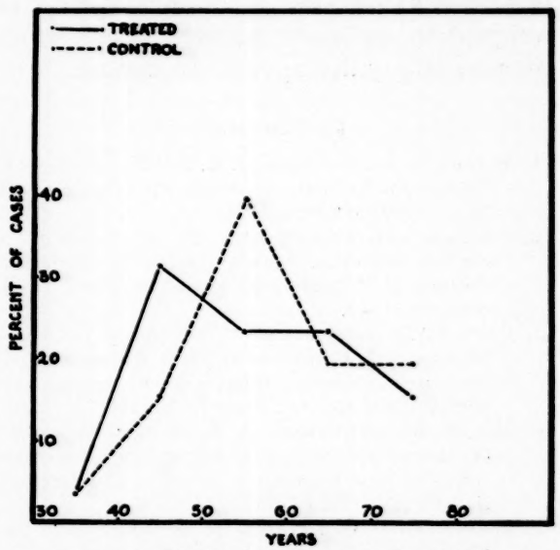

FIG. 9. The broken line represents the age distribution curve of the control group and the solid line represents the age distribution curve of the treated group in the series of Glueck, Strauss, Pearson and McGuire.[3]

SUMMARY

We should like to emphasize that this study was not designed to be critical of dicumarol or of any other type of therapy in myocardial infarction. Dicumarol therapy may be a distinct advance in the treatment of coronary thrombosis or even coronary sclerosis. It has, as an anticoagulant, at least certain theoretic advantages and possibly has additional pharmacologic actions as yet not described and little understood.

In our series of 414 cases of myocardial infarction 15.5 per cent died and 6.5 per cent had one or more thromboembolic phenomena. In 4,108 cases in the literature together with our 414 cases the combined mortality rate was 23.5 per cent and in 1927 cases from the literature together with our

414 cases embolic phenomena occurred in 10.8 per cent.

From the data presented it can be easily seen that age, sex, pre-existing hypertension, angina and previous infarction materially influence mortality. Therefore, the state of the heart at the time of infarction must be weighed in statistical analysis of the effectiveness of any therapeutic procedure.

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Effect of Orally Administered Dihydroergocornine (D.H.O. 180) on Hypertension*

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The purpose of this study was to evaluate the blood pressure lowering effects of orally administered dihydroergocornine (D.H.O. 180) in hypertensive individuals.

Dihydroergocornine (D.H.O. 180) was prepared by Stoll and Hoffmann[1] by dihydrogenation of the ergotoxine group of alkaloids. Dihydroergocornine, one of the derivatives, had marked sympatholytic and adrenolytic properties when given to animals. Dihydrogenation of the alkaloid[2,3,4] diminished or abolished the direct stimulating effects of ergocornine on smooth muscle.

Bluntschli and Goetz[5] reported that dihydroergocornine (D.H.O. 180) administered intravenously lowered the blood pressure in hypertensive patients. Fries, Stanton and Wilkins[6] achieved the same effect with intravenous administration and also orally in selected patients but the oral effective dose was roughly ten to twenty times greater than that of the intravenous dose.

MATERIALS AND METHODS

Ten hypertensive patients who had been attending the hypertensive clinic at Temple University Hospital for many months were selected. Of the ten patients eight were females and two were males. The age ranged from thirty-eight years to fifty-eight years with an average age of forty-eight years. Eye-ground studies of the ten patients revealed seven patients with a grade II sclerosis, two patients with a grade I sclerosis and one patient with only arteriolar attenuation. Of the seven patients with a grade II sclerosis six had attenuation, four had angiospasm, four had evidence of hemorrhages and one patient had exudates. Kidney function was determined by the Fishberg urine concentration test. Three patients (Nos. 1, 5 and 6) showed a concentration of less than 1.020. The medications prior to the oral dihydroergocornine (D.H.O. 180) therapy included phenobarbital (dosage ranging from 0.015 gm. to 0.030 gm., three to four times daily) and nicotinic acid (dosage ranging from 0.050 gm. to 0.1 gm., three to four times daily). In addition two patients (Nos. 5 and 7) were on potassium thiocyanate therapy; one patient (No. 5) was on a maintenance dose of digitalis (0.1 gm. daily) and one patient was on mannitol hexanitrate therapy (0.015 gm. four times daily). (Table I.)

A control blood pressure reading was taken weekly for ten weeks in a sitting position; the same arm and blood pressure apparatus were used in recording the weekly blood pressure readings throughout the entire observation. The lowest, the highest and the average blood pressure readings are recorded in Table I.

At the end of the tenth week the patients were placed on oral dihydroergocornine (D.H.O. 180) therapy and their previous medications discontinued with the following two exceptions, Case 5 who was maintained on maintenance doses of digitalis and potassium thiocyanate (blood level of 8 mg. per 100 cc.) and Case 7 who was also maintained on a maintenance dose of potassium thiocyanate (blood level of 10 mg. per 100 cc.). The oral D.H.O. 180 dosage ranged from 4 mg. daily to 12 mg. daily in divided doses. The patients were on oral D.H.O.

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180 therapy for six weeks; the lowest, the highest and the average blood pressure readings are recorded in Tables II and III.

Dihydroergocornine (D.H.O. 180) therapy was discontinued at the end of the sixth week. The patients were then given placebo tablets (thiamin chloride 2 mg.), which were similar in appearance to the dihydroergocornine tablets. The patients were under observation for four weeks and weekly sitting blood pressure readings taken. The average blood pressure readings are recorded in Table III.

During the fifth and sixth weeks after discontinuation of oral D.H.O. 180 the patients' blood pressure responses to intravenous administration of 500 mg. of tetraethylammonium chloride and also to 0.25 mg. of D.H.O. 180 were tested. The blood pressure readings prior to and after the intravenous administration were taken in the supine position. The blood pressure readings are recorded in Table IV and in Figures 1 and 2, respectively.

RESULTS

Prior to the oral administration of dihydroergocornine (D.H.O. 180) the average blood pressure reading of the ten hypertensive patients revealed a diastolic blood pressure ranging between 107 mm. of Hg and 130 mm. of Hg and a systolic blood pressure between 172 mm. of Hg and 224 mm. of Hg. The average blood pressure reading of the ten patients was 193/119. (Table III.)

During oral D.H.O. 180 therapy the average blood pressure reading of each of the ten hypertensive patients revealed a diastolic blood pressure ranging between

TABLE I

SUMMARY OF FINDINGS PRIOR TO THE ORAL DIHYDROERGOCORNINE (D.H.O. 180) THERAPY

No.	Name	Age	Sex	Eye-ground Findings	Urine Specific Gravity Test (Fishberg)	Medications Prior to Dihydroergocornine (D.H.O. 180) Therapy	Lowest Blood* Pressure	Highest Blood* Pressure	Average Blood* Pressure
1	P. F.	44	F	Sclerosis grade II, angiospasm, hemorrhages	1.005 1.010	Nicotinic acid, phenobarbital, aspirin	170/110	210/150	205/130
2	R. T.	52	M	Sclerosis grade II, attenuation, hemorrhages, exudates	1.018 1.020	Nicotinic acid	160/120	192/140	172/128
3	M. M.	38	F	Sclerosis grade II, attenuation, hemorrhages	1.011 1.020	Phenobarbital	184/110	208/130	191/116
4	M. D.	45	F	Sclerosis grade II	1.015 1.027	Phenobarbital, nicotinic acid	130/90	198/130	176/111
5	E. D.	43	F	Sclerosis grade II, attenuation, angiospasm	1.011 1.012	Digitalis, potassium thiocyanate, ammonium chloride, phenobarbital	198/108	230/140	208/117
6	B. T.	48	F	Attenuation	1.010 1.015	Phenobarbital	160/90	200/140	179/117
7	L. F.	51	F	Sclerosis grade I	1.017 1.020	Potassium thiocyanate	180/100	230/116	203/107
8	R. C.	58	F	Sclerosis grade II, attenuation	1.016 1.022	Mannital hexanitrate, phenobarbital	190/100	210/150	200/122
9	W. S.	51	M	Sclerosis grade II, attenuation, angiospasm	1.016 1.021	Phenobarbital	190/106	260/170	224/126
10	B. C.	52	F	Sclerosis grade I	1.018 1.023	Phenobarbital	150/100	180/130	172/116

* Sitting blood pressure readings

100 mm. of Hg and 130 mm. of Hg and a systolic blood pressure ranging between 145 mm. of Hg and 226 mm. of Hg. The average blood pressure reading of the 10 patients was 191/114. (Table III.) One patient (Case 1) developed a slight nasal congestion in the first week of therapy; another (Case 5) was hospitalized at the end of the third week of oral D.H.O. 180 therapy because of a cerebral vascular accident.

After oral D.H.O. 180 was discontinued the average blood pressure reading of each of the ten patients revealed a diastolic blood pressure ranging between 98 mm. of Hg and 127 mm. of Hg and a systolic blood pressure between 144 mm. of Hg and 212 mm. of Hg. The average blood pressure reading of the ten patients was 186/112. (Table III.) It is interesting to note that the average blood pressure level of the group while on thiamin chloride was lower than during the control period or the period during D.H.O. 180 therapy.

Prior to intravenous injection of 500 mg. of tetraethylammonium chloride the diastolic blood pressure ranged between 104 mm. of Hg and 140 mm. of Hg and the systolic blood pressure ranged between 150 mm. of Hg and 240 mm. of Hg. The average blood pressure reading of the ten patients was 203/126. After the intravenous administration of the drug the diastolic

TABLE II

DOSAGE, SYMPTOMS AND BLOOD PRESSURE* DURING ORAL DIHYDROERGOCORNINE (D.H.O. 180) THERAPY

No.	Name		Weekly Observations					
			1	2	3	4	5	6
1	P. F.	B. P. reading	200/124	214/124	200/116	180/116	194/122	212/120
		Dosage†	1	1	1	3	2	2
		Symptoms	N.C.	0	0	0	0	0
2	R. T.	B.P. reading	200/120	202/120	200/130	168/124	180/120	170/120
		Dosage†	1	1	1	3	2	2
		Symptoms	0	0	0	0	0	0
3	M. M.	B. P. reading	170/110	180/100	170/104	168/104	208/110	178/110
		Dosage†	1	1	1	3	2	2
		Symptoms	0	0	0	0	0	H
4	M. D.	B. P. reading	184/112	170/110	180/110	160/100	174/110	168/108
		Dosage†	1	1	1	2	2	2
		Symptoms	0	0	0	0	H-D	H-D
5	E. D.	B. P. reading	220/126	208/124	250/140	Hospitalized for cerebral hemorrhage		
		Dosage†	1	1	1			
		Symptoms	0	0	0			
6	B. T.	B. P. reading	170/110	160/100	160/100	184/124	212/120	164/110
		Dosage†	1	1	1	2	2	2
		Symptoms	0	0	0	H	0	0
7	L. F.	B. P. reading	210/112	190/100	206/110	194/104	184/112	204/110
		Dosage†	1	1	1	3	2	2
		Symptoms	0	0	0	T	H-D	0
8	R. C.	B. P. reading	210/130	194/110	190/110	200/114	204/110	190/124
		Dosage†	1	1	1	2	2	2
		Symptoms	0	0	0	0	H	0
9	W. S.	B.P. reading	230/120	190/100	200/120	240/120	208/110	220/120
		Dosage†	1	1	1	2	2	2
		Symptoms	0	0	0	0	0	0
10	B. C.	B.P. reading	150/106	130/88	140/100	150/104	146/94	150/100
		Dosage†	1	1	1	2	2	2
		Symptoms	0	0	H	0	0	0

* Sitting blood pressure readings.
† Number of mg. four times daily.
H—Headache
D—Dizziness
T—Tinnitus
N.C.—Nasal congestion

blood pressure ranged between 60 mm. of Hg and 126 mm. of Hg and a systolic blood pressure ranged between 80 mm. of Hg and 190 mm. of Hg. The average blood pressure reading of the ten patients was 142/97. All blood pressure readings were taken in the supine position.

All the individuals experienced the usual temporary drug symptoms (1) dryness of the mouth, (2) metallic taste, (3) generalized tingling and (4) blurred vision. The maximum fall in blood pressure was recorded within 120 seconds after the intravenous administration of the drug. One patient (Case 2) had a drop in the systolic blood pressure of 48 mm. of Hg but the diastolic blood pressure remained at 126 mm. of Hg prior to and after the intravenous administration of 500 mg. of tetraethylammonium chloride.

The blood pressure readings were taken in the supine position prior to and after the intravenous administration of 0.25 mg. of D.H.O. 180. Prior to administration the diastolic blood pressure ranged between 110 mm. of Hg and 146 mm. of Hg and the systolic blood pressure ranged between 170 mm. of Hg and 242 mm. of Hg. The average blood pressure reading of the ten patients was 205/125. After administration the diastolic blood pressure ranged between 94 mm. of Hg and 120 mm. of Hg and the systolic blood pressure ranged between 134 mm. of Hg and 198 mm. of Hg. The average blood pressure reading of the ten patients was 171/106. (Table IV.) The blood pressure lowering effect of the intravenous D.H.O. 180 was not as marked as that resulting from the intravenous administration of tetraethylammonium chloride. The maximum fall in the blood pressure was recorded between ten and thirty minutes after the intravenous administration of the drug. The duration of the response was greater with the intravenous administration of D.H.O. 180 than with the tetraethylammonium chloride, the duration of the response ranged from 30 to 120 minutes. All ten patients gave some response to the intravenous administration of D.H.O. 180 and three patients (Nos. 3, 5 and 10) developed a side reaction of a mild nasal stuffiness or congestion which was temporary.

COMMENT

A definite drop in the blood pressure was noted in all ten patients following the intravenous administration of dihydroergocornine (D.H.O. 180)* but none of the patients showed a significant drop in the blood pressure during oral D.H.O. 180 therapy. It is also interesting to note that three patients developed nasal stuffiness after the intravenous administration of D.H.O. 180 but only one patient in the first week of oral D.H.O. 180 therapy had a suggestion of nasal stuffiness. Since all ten patients had some response both to the intravenous administration of tetraethylammonium chloride and to D.H.O. 180, one would expect that an oral dosage of D.H.O. 180, approximately twenty to fifty times greater than the effective intravenous dose, should have shown some effect if it were absorbed. We must, therefore, suspect either (1) inadequacy of absorption of the orally administered D.H.O. 180 from the gastrointestinal tract or (2) inactivation of

TABLE III
BLOOD PRESSURE* RESPONSE TO ORAL DIHYDROERGOCORNINE (D.H.O. 180)

No.	Name	Average Blood Pressure Reading Prior to D.H.O. 180 Therapy	Average Blood Pressure Reading during D.H.O. 180 Therapy	Average Blood Pressure Reading after Discontinuation of D.H.O. 180 Therapy
1	P. F.	205/130	220/120	195/127
2	R. T.	172/128	187/122	185/110
3	M. M.	191/116	179/106	181/107
4	M. D.	176/111	172/110	175/108
5	E. D.	208/117	226/130	207/124
6	B. T.	179/117	171/110	182/116
7	L. F.	203/107	198/108	204/112
8	R. C.	200/122	198/116	172/112
9	W. S.	224/126	215/115	212/120
10	B. C.	172/116	145/100	144/98
	Average	193/119	191/114	186/112

* Sitting blood pressure readings.

* The dihydroergocornine was generously supplied by the Sandoz Chemical Works, Inc., New York.

the D.H.O. 180 within the gastrointestinal tract.

It is now fairly well known and accepted that dihydroergocornine (D.H.O. 180) has adrenolytic properties. However, since several reports in the literature indicate that it is also sympatholytic and that this effect is exerted at a higher level in the central nervous system, it seemed advisable to check some of the properties of this drug in trained unanesthetized dogs. For this purpose five dogs were accustomed to direct arterial punctures for taking blood pressure readings and to simultaneous intravenous injections into the hind leg. Blood pressure readings were recorded by means of a membrane manometer. The control response of each dog to epinephrine (2 microgram per kg.) was measured at intervals and all control responses were in the range of 25 to 46 mm. of Hg. Subsequently, the responses to epinephrine were measured after intravenous and oral doses of D.H.O. 180. The results of these procedures are shown in

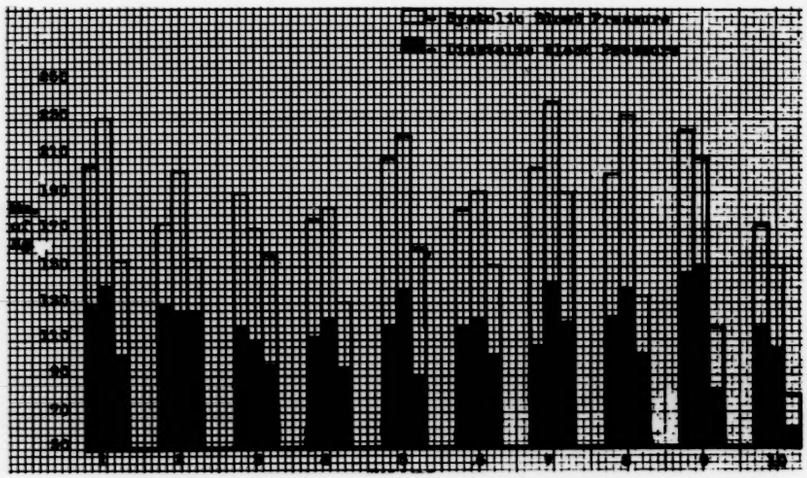

FIG. 1. Blood pressure response to intravenous tetraethylammonium chloride. The first column represents the average sitting blood pressure reading, the second column the blood pressure reading prior to intravenous tetraethylammonium chloride and the third column the blood pressure reading after 500 mg. of intravenous tetraethylammonium chloride (second and third readings are supine readings).

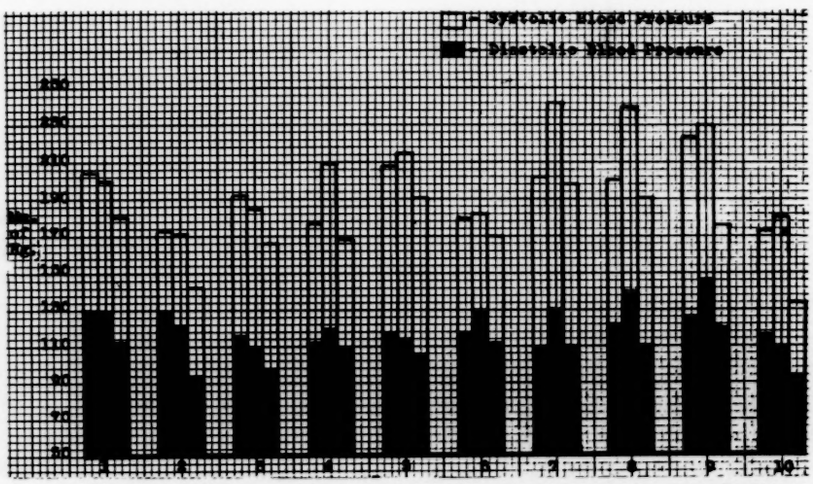

FIG. 2. Blood pressure response to intravenous dihydroergocornine (D.H.O. 180). The first column represents the average sitting blood pressure reading, the second column the blood pressure reading prior to the intravenous D.H.O. 180 and the third column the blood pressure reading after 0.25 mg. of intravenous D.H.O. 180 (second and third readings are supine readings).

Figure 3. It has been clearly shown that oral administration is much less effective than intravenous; although the dose, when compared on a weight-to-weight basis is much larger than that given to hypertensive patients; it exhibits very little adrenolytic effect. It also appears that in the unanesthetized, trained dogs one must give at least 0.1 mg. per kg. of D.H.O. 180 intravenously before adrenolytic effects are complete. When D.H.O. 180 demonstrated some adrenolytic effect following oral administration, the effect appeared after one and one-half to two hours and persisted for some time.

TABLE IV
BLOOD PRESSURE RESPONSE TO INTRAVENOUS ADMINISTRATION OF DIHYDROERGOCORNINE (D.H.O. 180)

No.	Name	Blood Pressure Reading (Supine) before Intravenous D.H.O. 180	Blood Pressure Reading (Supine) after Intravenous D.H.O. 180 (0.25 mg.)
1	P. F.	200/130	180/112
2	R. T.	170/120	142/94
3	M. M.	184/110	166/98
4	M. D.	210/120	160/110
5	E. D.	214/114	190/104
6	B. T.	180/130	170/112
7	L. F.	242/130	198/110
8	R. C.	240/140	190/110
9	W. S.	230/146	176/120
10	B. C.	180/110	134/94

In the acute type of experiment it can be demonstrated that a reduction in the pressor response to clamping of the carotid arteries occurs after doses as low as 0.02 mg. per kg. This difference in dosage levels for the production of adrenolytic and sympatholytic effects may indicate that the mechanisms involved are entirely different, particular since they are in the reverse order from those found for other agents such as dibenamine. This difference in mechanism is also indicated by the report of Rothlin,[3] more recently confirmed by Bluntschli and Goetz,[5] that the sympatholytic action is exerted at some higher level in the central nervous system rather than at the sympathetic terminals.

SUMMARY AND CONCLUSIONS

1. We were unable to demonstrate any significant blood pressure lowering effects in ten hypertensive patients given dihydroergocornine (D.H.O. 180) orally. The oral dosage used was approximately twenty to fifty times greater than the effective intravenous dose.

2. All ten patients gave some response (blood pressure lowering effects) to the intravenous administration of D.H.O. 180. Three of the patients developed a side reaction of a temporary mild nasal stuffiness or congestion.

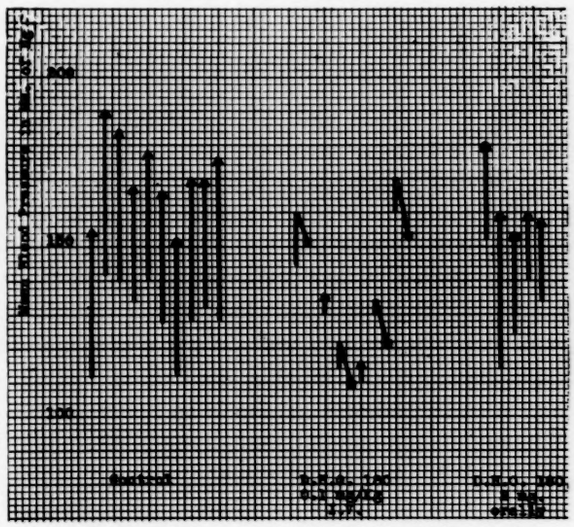

FIG. 3. Epinephrine responses in unanesthetized dogs.

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Review

Digitalis Poisoning*

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DIGITALIS is one of the most valuable drugs in our armamentarium, not only because of the frequent need for it but also because of the remarkably efficacious effects when it is properly employed. Since its introduction to the profession in 1785 by William Withering the drug has been widely but not always wisely used. Like most potent drugs possessing definite therapeutic properties, digitalis is dangerous if used incautiously and under such circumstances is capable of producing deleterious effects. This is particularly true of the highly potent pure glycosides now in common use, especially when they are injected intravenously. In this paper the term "digitalis" is used to indicate not only the crude drug but also the pure glycosides derived from it as well as other digitalis-like drugs. All have qualitatively similar actions.

Withering recognized and described the foxglove as having the ability to cause " . . . sickness, vomiting, purging, giddiness, confused vision, objects appearing green or yellow; increased secretion of urine, with frequent motions and sometimes inability to retain it; slow pulse, even as slow as 35 in a minute, cold sweats, convulsions, syncope, death."[1] He also observed that " . . . it was found very possible to pour in an injurious quantity of the medicine, before any of the signals of forebearance appeared."[2]

The rational use of digitalis is based on its physiologic effects and, even though little has been learned of the basic biochemical mechanism whereby the drug produces its effects, the principles governing the indications for its use as well as the concepts of its proper employment seem well established.[3] The cardiac glycosides are the active principles in digitalis and digitalis preparations, and the cardiac musculature is more sensitive to these principles than other tissues. It is believed that the glycosides enter the myocardial protoplasm and are split there, in some manner, to release the active aglycones. The aglycones, in turn, apparently produce certain direct changes on the heart muscle fibers.[4]

First, the myocardium is stimulated, resulting in an increase of the systolic force of contraction (systolic tension) and a shortening of the period of systole. Stewart et al.[5] interpret the results of their studies as showing that digitalis reduces the heart size in both normal and pathologic hearts and that the cardiac output consequently varies, depending on the initial size of the heart. Thus according to this concept, in the normal person digitalis reduces the normal size of the heart so that it becomes too small for the normal vascular system and, in turn, the efficiency of the heart as a pump is impaired. This is reflected in a slight reduction of the cardiac output. In the dilated, failing heart, on the other hand, digitalis reduces the heart toward normal in relation to the vascular system and enables the work per beat to be increased so that the cardiac output rises, with a resulting increase in the blood velocity, shortening of the circulation time and diminution of the arteriovenous oxygen difference. Mechanical efficiency of the heart is increased as evidenced by a diminished oxygen consumption.[4] Venous pressure if elevated is reduced[6] and as the circulation improves, renal filtration is enhanced, diuresis is promoted, excess so-

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dium is excreted and the blood volume is decreased.

Second, the refractory phase is prolonged and conduction is slowed through the auriculoventricular bundle. Evidence of this is frequently furnished by prolongation of the P-R interval in the electrocardiogram. However, this phenomenon can best be observed clinically in auricular fibrillation for the impulses from the auricles can be slowed and the number transmitted through the bundle to the ventricles can be materially reduced by this blocking action of digitalis in appropriate doses. When small doses of the drug are employed, sufficient only to improve myocardial tone, the ventricular slowing is mostly secondary to improved circulation and is largely governed by the relatively increased vagal tone for atropine will abolish the vagal activity and allow the ventricular rate to increase again. But as the dose of digitalis is increased, the direct action of the drug on auriculoventricular conduction becomes increasingly important and the control of the ventricular rate passes proportionately from the vagal to the extravagal mechanism, for the administration of atropine becomes less and less effective in increasing the ventricular rate.[7]

Third, as conduction through the normal pathways is progressively slowed, digitalis causes the myocardium to become increasingly irritable. Ectopic foci appear and initiate abnormal impulses, most often detected by the presence of premature beats. If the irritability is increased to a sufficient degree, ectopic mechanisms may entirely replace normal impulse initiation and conduction. This effect of digitalis is not usually sought in therapy and its development may ultimately lead to fatal ventricular fibrillation in severe poisoning.

INDICATIONS FOR DIGITALIS

The primary indications for digitalis may be deduced from the foregoing discussion. The presence of congestive failure, whether of predominantly right- or left-sided type, is the chief condition calling for digitalis therapy. Digitalis is employed in auricular fibrillation and flutter with the intention of slowing the ventricular rate by means of increasing the auriculoventricular block. Digitalis may be indicated to prevent recurrence of failure in patients in whom it has once occurred[8,9] and use of the drug is justified in early left ventricular failure or impending failure, indicated by paroxysmal nocturnal dyspnea or dyspnea on slight exertion.[10] However, since it is desirable to reserve digitalis until it actually is needed, it is well to remember and evaluate properly the importance of reduction of activity and salt restriction.[10]

Because of the frequent unwarranted use of digitalis in certain conditions, Broome and Orgain[11] have listed such situations as "erroneous indications" for digitalis. This group includes such conditions as heart murmurs, routine preoperative preparation, neurocirculatory asthenia, constrictive pericarditis, angina pectoris, coronary occlusion, thyrotoxicosis, shock, toxemia and acute rheumatic fever. Sino-auricular tachycardia, anxiety attacks with hyperventilation and non-cardiac edema are worthy of being added to this list of "erroneous indications." The routine use of digitalis preoperatively deserves special comment because it is not only unwarranted[12] but also dangerous. The need for digitalis during anesthesia and operative procedures must be critically evaluated, as Greene[13] has forcefully pointed out in his discussion of a case[14] in which a digitalis preparation was used in the attempt to decrease tachycardia under anesthesia, with the result that nearly fatal ventricular fibrillation was produced. Yet all too often one hears the question of preoperative digitalization dismissed with the fallacious statement that "in equivocal cases digitalis may be of value, but even if it is not, the drug will do no harm!"

CONTRAINDICATIONS TO DIGITALIS

The contraindications to digitalis are not numerous, the chief one being the absence of definite indications for the use of the drug. Evidence of digitalis poisoning is the

next important contraindication. Digitalis is contraindicated in paroxysmal ventricular tachycardia because it enhances the myocardial irritability and may precipitate ventricular fibrillation. In certain cases of Morgagni-Adams-Stokes syndrome with high grade block, digitalis may be contraindicated because of the increased danger of sino-auricular or auriculoventricular block during caroticovagal stimulation.[15,16] The presence of intraventricular block calls for particular caution in the use of the drug because if sufficient digitalis is given to lead to premature ventricular beats the danger of ventricular fibrillation is increased.[17] The increased hazard of such a circumstance is illustrated electrocardiographically by Tandowsky et al.[18] in a case resulting in a fatality. Digitalis is not the drug of choice in paroxysmal auricular fibrillation unless one is willing to accept chronic auricular fibrillation which may result from its use. Also, in the Wolff-Parkinson-White syndrome digitalis may bring on attacks of paroxysmal tachycardia of supraventricular origin. Quite naturally, the drug is contraindicated in instances of allergic reaction to digitalis antigens but such cases are rare.[15,19]

The presence of hypertension, hypotension, coronary artery disease, angina, infarction, auriculoventricular block, myocarditis, sinus tachycardia or bradycardia, premature ventricular beats or other rhythm disturbances does not in itself constitute contraindications to the use of the drug. However, it is true that in some of these conditions digitalis must be used with more than the usual amount of caution. Furthermore, considerable variation in the response of congestive failure to digitalis may result when the failure is complicated by various conditions. In general when complicating factors are secondary to heart failure, digitalis is effective in restoring these factors toward normal; but the drug is of more limited value when failure itself is secondary to a primary condition, e.g., active myocarditis. Thus in cases of congestive failure with sinus tachycardia, digitalis in therapeutic amounts usually slows the heart rate, secondarily for the most part, to the restoration of compensation. On the other hand, the degree of slowing of a rapid rate by digitalis is frequently disappointing, even in cases of auricular fibrillation, if the tachycardia is due to some toxic reaction (thyrotoxicosis, diffuse or focal myocarditis) rather than to some degree of decompensation. The drug is seldom of benefit in the absence of failure.

MATERIAL

Although the foregoing facts have been well publicized to the profession, the use of digitalis continues to be abused and poisoning with the drug occurs all too frequently.[2] We recently have observed seven instances of rather severe digitalis poisoning within a period of seven months. These cases have come to our attention through routine hospital and clinic duties without any particular effort being made to collect or uncover such cases. While this incidence is not necessarily surprising, it prompted us to consider why digitalis intoxication occurs as frequently as it does. With this in mind the records of cases with the diagnosis of digitalis poisoning in Duke Hospital during the years 1939 to 1945, inclusive (a seven-year period), have been reviewed. A few cases were excluded because it was felt that the evidence in these records was insufficient to justify the diagnosis. However, twenty-eight cases in which the diagnosis appeared to be correct were thus collected. To these twenty-eight have been added the seven cases recently observed by us to make a total of thirty-five, forming the group for the present discussion. The ages of the patients studied ranged between fifteen and eighty-seven years. There were nineteen males and sixteen females. Of the thirty-five, thirty were white and five were colored. On the other hand, it is recognized that many cases of digitalis intoxication were lost from collection because the diagnosis was never listed as such. This error occurs sometimes through misinterpretation of the clinical findings but probably more often through oversight.

History of digitalis administration is useful but of limited value in making the diagnosis of digitalis poisoning. In the thirty-five cases collected there was a reasonable story of digitalis medication in the history of all although the amount and time of such therapy were quite variable. Some patients were not aware of having taken digitalis although their descriptions of the medication, of its mode of administration and of its effects were such that it was justifiably presumed to be a digitalis preparation. In other cases the history of the amount and time of administration was quite specific and it was possible to determine exactly how much digitalis had been given and when. Among those cases in which the specific amounts and times of digitalis administration were known, many (eighteen patients) were found to have received doses significantly exceeding the usual average dose. It was not possible in all cases to determine which preparation had been used. In twenty-four cases chiefly digitalis leaf had been given, in at least two cases digitoxin had been administered, and one patient took strophanthus and squill in a proprietary medicine in addition to his regular dosage of digitalis leaf.

CASE REPORTS

The following case reports are presented as illustrative of many of the factors leading to and influencing the course of digitalis poisoning.

CASE I. A fifty-four year old white female was referred to the Medical Out-Patient Clinic for cardiac evaluation. There was a history of rheumatic polyarthritis twenty-nine years previously. A letter from the patient's physician described an episode of cardiac decompensation about five weeks prior to her visit in which the patient suffered from dyspnea, orthopnea, ankle edema and had a pulse rate of 136. According to his letter, the physician gave one hypodermic injection of digitalis on the first day he was consulted, followed by three similar injections the following day. He then prescribed 1.5 gm. of digitalis orally over a period of six days, followed by 0.1 gm. daily thereafter. Two days after therapy began the patient was "fairly well compensated" and seven days later the pulse rate was noted to be 60 per minute by the physician. Twelve days after therapy began a pulse rate of 42 per minute was noted and the patient "was not able to see nearly so well" although there had "been no signs of overdigitalization."

On physical examination in the clinic, mitral systolic and diastolic murmurs were detected and the cardiac rhythm was found to be irregular with a rate of 96 per minute. An electrocardiogram showed auricular fibrillation with a ventricular rate of 100, frequent premature ventricular beats producing runs of bigeminy, and inversion of $T_{1, 2}$ and $_4$.

Diagnoses of inactive rheumatic heart disease, compensated, with mitral stenosis and insufficiency, auricular fibrillation and digitalis poisoning were made. A low salt diet was prescribed, restricted activity was advised, and digitalis was withheld until signs and symptoms of toxicity disappeared.

Comment. This case illustrates two of the common errors leading to digitalis poisoning. First, the drug is frequently prescribed by an unvarying system of dosage on a set schedule regardless of the patient's reaction and, second, when signs of poisoning appear they either are not recognized, are not appreciated or are ignored. Digitalis effectively slows the ventricular rate in most cases of auricular fibrillation by increasing the refractory period of the auriculoventricular node and establishing auriculoventricular block, thus allowing better ventricular filling and producing stronger systolic contraction. When the pulse abated to 60 and then to 42 per minute, the physician, although alert enough to make the observation, apparently was not aware of the danger and probability of complete heart block for he neglected to withhold the drug. By the time the electrocardiogram was taken the conducting ability of the AV node was depressed and the myocardial irritability was increased to the point that ectopic pacemakers (as evidenced by frequent premature ventricular beats with bigeminy) were activated.

CASE II. A fifty-two year old white female was admitted to the hospital because of ana-

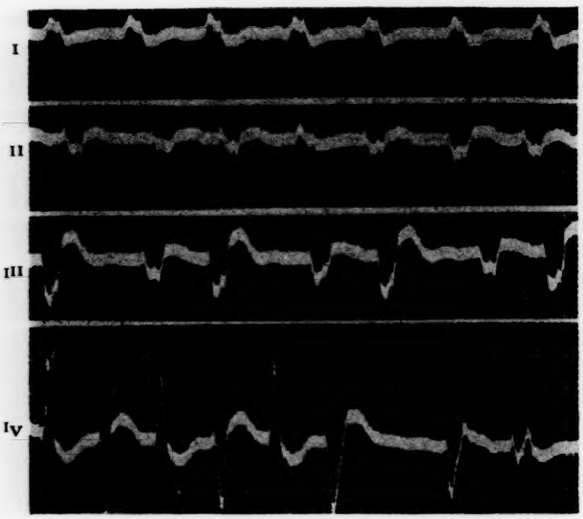

FIG. 1. White female, fifty-two years of age, with anasarca and history of taking digitalis every four hours for the preceding three weeks. Electrocardiogram on admission showing auricular fibrillation; IV conduction defect of left bundle type, premature ventricular beats producing runs of bigeminy, low voltage in limb leads and slurring of QRS complexes; ventricular rate: 107/min. Note STT deviations and short Q-T intervals. History of previous digitalis apparently was discredited or ignored, for rapid digitalization was attempted. Sudden death occurred six and one-half hours after admission.

sarca. About one year prior to admission the patient complained of dyspnea on exertion and weakness, which was treated by two weeks of bed rest in a sanatorium. Daily doses of an unknown quantity of digitalis were taken thereafter and the patient temporarily improved. About seven months later she again developed dyspnea and weakness as well as orthopnea and edema of the left ankle. She again was institutionalized in the sanatorium for five weeks of bed rest but she failed to improve, so she returned home where she remained at bed rest and continued to take digitalis daily. For five weeks prior to admission she became progressively more edematous and noticed palpitation of the heart. For the three weeks preceding her admission to the hospital the patient took "digitalis every four hours."

Physical examination revealed a white female with signs of anasarca. The blood pressure was 130/110 and the pulse rate was 115 per minute with complete arrhythmia. The heart was enlarged to the posterior axillary line; a systolic blow replaced the second sound at the apex and was transmitted over the precordium; the heart sounds were of poor quality; and cardiac pulsations were weak. Tendon reflexes were not elicited. X-ray of the chest showed clouding in both lung bases and an enlarged heart in all diameters. There was 2+ albuminuria. An electrocardiogram (Fig. 1) showed auricular fibrillation and an intraventricular conduction defect with premature ventricular beats, at times producing runs of bigeminy. The ventricular rate was 107 per minute. There was low voltage in the limb leads and slurring in all.

Apparently the electrocardiographic findings were misinterpreted or ignored at this time because, in spite of definite cardiographic warning signs and notwithstanding the history of recent (and probably tremendous) digitalis dosage, rapid digitalization was attempted. Digitalis, 0.2 gm., was given by mouth and forty-five minutes later 1 cc. salyrgan was given intravenously. An hour and a half after the first dose of digitalis was given, 0.2 gm. orally, it was repeated but the patient vomited. Three and a half hours after the initial dose of digitalis 2 "cat units" of digifolin® were given hypodermically; this was repeated two hours later. According to the record "good diuresis had begun" for the patient was on the bed pan when she unexplainably began to "gasp and became apneic." Thereupon she expired suddenly, six and a half hours after admission.

A complete autopsy was performed and the significant findings were an enlarged and diffusely hyperplastic thyroid, cardiac hypertrophy and dilatation, chronic passive congestion of the viscera and anasarca with hydrothorax and hydroperitoneum.

Comment. Several errors were committed in the hospital management of this case. First, rapid digitalization, including parenteral administration, entailed an unwarranted risk in such an individual, particularly in view of the history of presumably large doses of digitalis shortly before admission. Second, although the usual signs and symptoms of digitalis poisoning were not elicited, the complaints of weakness, palpitation and the finding of arrhythmia and progressive anasarca (in the face of more than adequate digitalis) apparently were ignored. Third, although an electrocardiogram was taken, it was not properly utilized or heeded before the potentially dangerous procedure of rapid digitalization was instituted. Fourth, in this case intra-

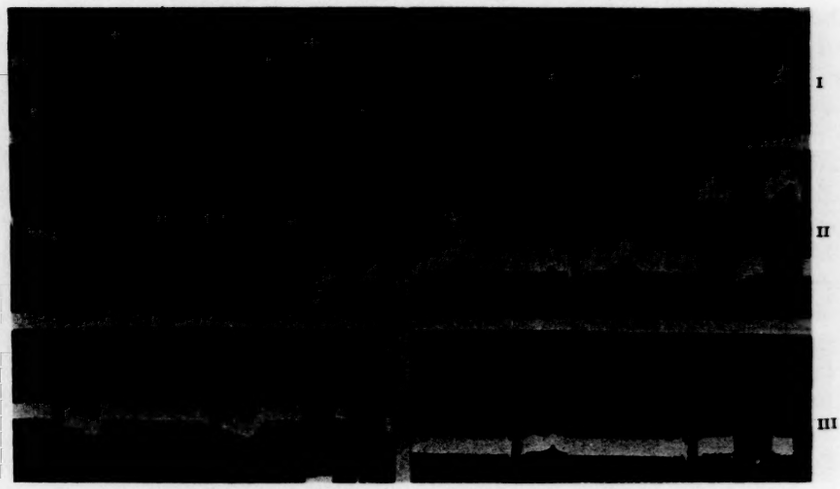

FIG. 2. White male, twenty-five years of age, with nervousness, lassitude and depression. A, electrocardiogram on second hospital day showing partial A-V heart block with dropped beats and S-T segment depression. These findings finally led to discovery of ingestion (at first denied) of digitoxin 0.2 mg. daily for preceding eight weeks. B, electrocardiogram within normal limits six weeks after discontinuance of digitoxin.

venous administration of a mercurial diuretic might be expected to play a significant part in increasing digitalis effects. The explanation usually offered for this phenomenon is the reabsorption into the blood stream of fluid containing digitalis from the tissue spaces and serous cavities.[20] The description of the manner of death and the paucity of autopsy findings lead one to assume that the immediate cause of the sudden death was ventricular fibrillation due to digitalis intoxication.

CASE III. A twenty-five year old white male was admitted to the hospital for general physical evaluation complaining chiefly of nervousness and feeling tired and depressed. Physical examination revealed no remarkable findings except for a systolic murmur, loudest at the pulmonic area, and a cardiac arrhythmia. An electrocardiogram (Fig. 2A) taken on the second hospital day revealed P-R interval 0.14 to 0.34 and was interpreted as showing partial heart block with frequent dropped beats. ST segments were depressed.

Although the patient at first denied taking any medication, requestioning led to the admission that he had taken a white tablet each day for two months, which "might be" for the heart. A letter from the family physician was received later which verified the suspicion of digitalis poisoning. Digitoxin, 0.2 mg., daily had been prescribed eight weeks prior to admission "because of a fast pulse rate, 86 to 90, and easy fatigue."

The patient was advised to discontinue the medication. A subsequent electrocardiogram six weeks later (Fig. 2B) was within normal limits.

Comment. The principal error committed in this case was, of course, that digitalis was prescribed when it was not indicated. It is interesting to note that none of the well known clinical signs or symptoms of digitalis poisoning were detected although a dangerous amount of the drug had accumulated in the myocardium. Because digitalis was not indicated and because the usual signs and symptoms of intoxication were absent, digitalis poisoning was not suspected at first. This case further illustrates that, even in the absence of initial digitalizing doses, so-called maintenance doses may cumulate to full digitalization in time, and even to intoxication if continued long enough.

CASE IV. A fifteen year old white male was admitted to the hospital with edema and dyspnea of two months' duration. There was a history of rheumatic fever fifteen months previously. Physical examination revealed a systolic

murmur at the apex of the heart, rales in the lung bases and edema of the ankles. Rheumatic heart disease with mitral insufficiency and cardiac insufficiency was diagnosed. Treatment with diuretics and a salt-poor diet was instituted and digitalization was accomplished by means of 2.8 gm. of digitalis in twelve days. An electrocardiogram on the third hospital day showed P-R interval 0.19, and diphasic T waves in leads II and III. After twelve days in the hospital the patient was discharged edema-free and was given a prescription for "digitalis 0.1 gm. *q.d.*"

Five days later the patient returned to the clinic complaining of nausea and vomiting. Questioning revealed that he had been taking digitalis, 0.1 gm., four times a day due to the pharmacist's error in copying the directions of the prescription. The "0.1 gm. *q.d.*" had been interpreted to mean 0.1 gram *q.i.d.* An electrocardiogram showed auricular fibrillation with frequent premature ventricular beats. The patient was instructed to resume 0.1 gm. of digitalis daily.

Eight months later he was readmitted with a history of increasing edema for three weeks. Occasional nausea had been corrected by skipping doses of digitalis in the two months prior to readmission. In the hospital the patient was given 1.7 gm. of digitalis in seven days and the ventricular rate abated from 104 to 86. However, the cardiac decompensation was not relieved although further maintenance with 0.1 gm. of digitalis daily was continued in spite of nausea. On the twelfth hospital day the patient suddenly expired with what was thought to be a pulmonary embolus.

Comment. This case illustrates a hazardous method of writing the directions in a prescription. Abbreviations or symbols which are likely to be confused with one another should be recognized as potentially dangerous and, if employed in directions, they should be very carefully written so that no possibility of misinterpretation by the pharmacist can occur. The failure of digitalis to control decompensation in active carditis also is illustrated. Evaluation of the degree of digitalization and its maintenance is most difficult in this type of case.[21]

CASE V. A fifty-nine year old white male was admitted to the hospital because of weight loss, weakness and anorexia for three weeks. He had been known to have hypertension for ten years. Eight months prior to admission he noticed dyspnea and six months later orthopnea and ankle edema developed. Six weeks before entering the hospital his family physician informed him that his heart was enlarged and treated him with 1.2 mg. of digitoxin followed by 0.2 mg. daily thereafter. His edema and dyspnea disappeared in about two weeks but a week later he began to suffer from weakness, anorexia, weight loss and diarrhea. For about a week prior to entering the hospital the patient had failing vision, staggering gait, drowsiness, mental confusion, nausea and vomiting.

Physical examination revealed a disoriented individual with hypertension and cardiac enlargement, a systolic and diastolic aortic thrill and auricular fibrillation. An electrocardiogram (Fig. 3A) showed auricular fibrillation with a ventricular rate of 40 and ST segment sagging in all leads.

Digitalis was withheld. On the fourth hospital day another electrocardiogram showed very little change but by the eighth day the patient's sensorium was clear and his appetite was returning. Fifteen days after admission an electrocardiogram (Fig. 3B) showed a rate of 55 with sinus rhythm, P-R interval 0.20, diphasic T1 and 2, and depressed T3 and 4. Although he felt well, some ankle edema recurred, so on the sixteenth and seventeenth days he was given 1.4 mg. of digitoxin, followed by 0.2 mg. daily. On the eighteenth hospital day a salt-free diet finally was instituted and on the twenty-second day digitoxin was reduced to 0.1 mg. daily. An electrocardiogram (Fig. 3C) on the twenty-fourth day showed a persistence of the sinus rhythm with a rate of 65 but T3 and 4 were more depressed. He was discharged the next day, edema-free, but he was given instructions to remain under observation on a salt-free diet and 0.1 mg. of digitoxin daily.

Comment. This case is an example of the production of auricular fibrillation by digitalis. There were ample signs and symptoms indicating digitalis poisoning, yet they apparently were not recognized or, if so, they were ignored in spite of the fact that the therapeutic effects of the drug had been definitely manifested prior to the toxic effects. Furthermore, the value of salt restriction was overlooked until late in the therapeutic regimen. Because of this the

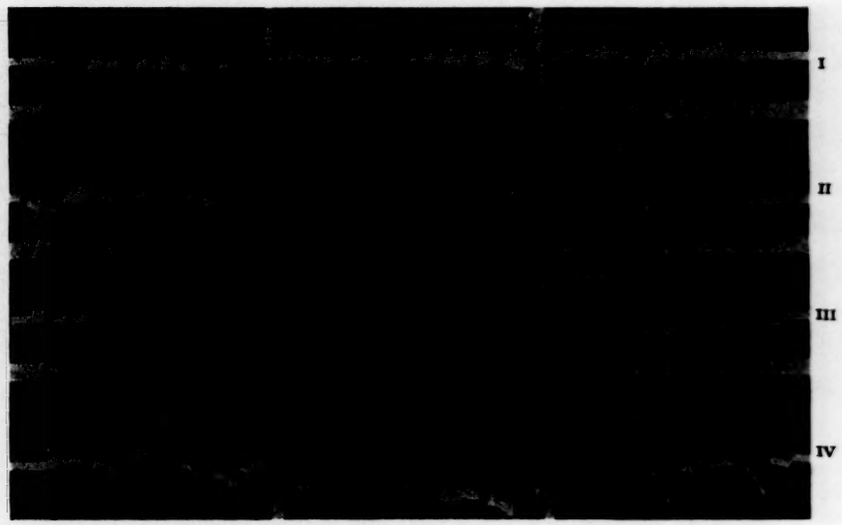

FIG. 3. White male, fifty-nine years of age, with weight loss, weakness and anorexia; history of 1.2 mg. digitoxin six weeks prior to admission followed by 0.2 mg. daily. A, electrocardiogram on admission showing auricular fibrillation with a ventricular rate of 40/min. and S-T segment sagging in all leads. B, electrocardiogram fifteen days after discontinuance of digitoxin showing reversion to sinus rhythm. C, electrocardiogram nine days later, during which time 2.5 mg. digitoxin were administered, showing increased depression of S-T segments in leads III and IV.

recurrent failure required redigitalization and maintenance with the same dosage that had been toxic previously until the use of a salt-free diet resulted in a reduction of the amount of digitalis required to control the decompensation.

CASE VI. A sixty year old white female was admitted to the hospital with complaints of headaches, nausea and vomiting. There was a history of dyspnea and orthopnea eight weeks prior to admission. Her physician treated her with one digitalis tablet three times a day for three days followed by one twice a day thereafter. Five weeks later headache, nausea and vomiting developed. Thereupon she went to a hospital where digitalis was discontinued for four days. When discharged she was instructed to take one digitalis tablet twice a day for two days, followed by one each day for seven days. However, under this regimen the headache recurred. The patient's physician then increased the digitalis to one tablet three times a day for three days, followed by one tablet twice a day. The severity of the headache increased and nausea and vomiting recurred.

Physical examination on admission revealed findings of arteriosclerosis and cardiac dilatation, with occasional premature beats. An electrocardiogram showed rate 102, P-R interval 0.20, frequent premature ventricular beats with short runs of sino-auricular block and T wave inversion in leads II, III and IV. Digitalis was withheld and five days later an electrocardiogram (Fig. 4A) showed rate 142, P-R interval 0.16, frequent premature auricular beats with short runs of paroxysmal auricular tachycardia and sagging of ST segments in leads II, III, and IV. Seven days after admission the patient was given potassium chloride, 1.0 gm. t.i.d., and the following day the electrocardiogram (Fig. 4B) showed a rate of 100 and disappearance of the premature auricular beats. Potassium chloride was discontinued on the ninth day; the next day the electrocardiogram (Fig. 4C) showed reappearance of the premature auricular beats. They were still present two days later at time of discharge from the hospital. Eight days after discharge the electrocardiogram revealed sino-auricular tachycardia with occasional premature ventricular beats. Fourteen days later (Fig. 4D) the rate was 90, the P-R interval was 0.16 and only occasional premature auricular beats were present. The T waves were upright and there was no sagging of ST segments.

The patient received no more digitalis. When

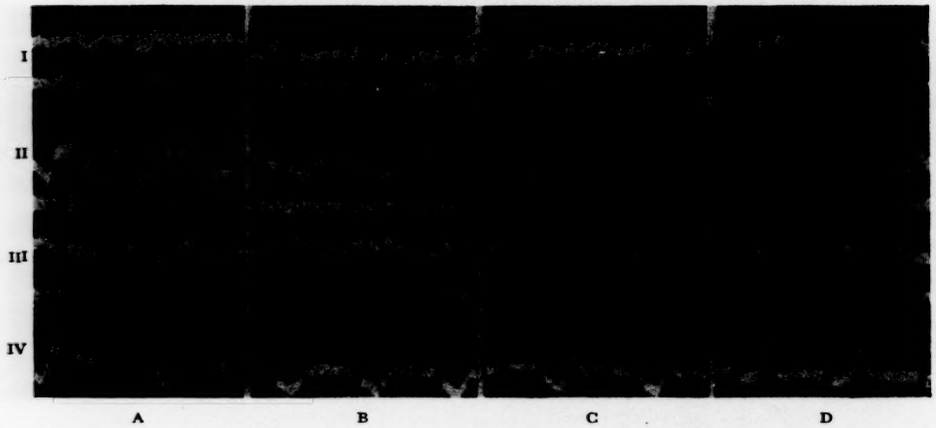

FIG. 4. White female, sixty years of age, with headaches, nausea and vomiting; history of digitalis ingestion varying between 1 and 3 tablets per day for eight weeks prior to admission. A, electrocardiogram five days after admission and discontinuance of digitalis showing frequent premature auricular beats with short runs of paroxysmal auricular tachycardia and STT complex changes characteristic of digitalis. B, electrocardiogram three days later while KCl 1.0 gm. t.i.d. was being administered, showing disappearance of the premature auricular beats. C, electrocardiogram ten days after digitalis withdrawal and one day after supplementary KCl was discontinued, showing a reappearance of the premature auricular beats. D, electrocardiogram one month after digitalis withdrawal showing normal STT complexes and only occasional premature auricular beats.

next seen two years later there was no cardiac arrhythmia although a recent history suggestive of paroxysmal nocturnal dyspnea was elicited. A salt-poor diet was prescribed and four months afterward her complaints had disappeared.

Comment. This patient had ample symptoms of digitalis poisoning well correlated with the history of digitalis ingestion and yet the drug was pushed persistently to dangerous limits, as evidenced by increased myocardial irritability.

The effect of potassium on the digitalis-poisoned heart is also illustrated in this case. Friedman and Bine[22] have recently produced evidence to show that excess potassium inhibits the action of digitalis on the heart partly by intrinsically depressing the irritability of the heart and partly by replacing the potassium lost from the heart when it is exposed to toxic amounts of digitalis. Loss of potassium from the heart probably results only when therapeutic amounts of digitalis are exceeded and toxic effects appear.

Furthermore, this case exemplifies the fact that some patients with mild congestive failure can be controlled better by sodium restriction without the use of digitalis.

CASE VII. A seventy-two year old colored male was seen in the Out-Patient Clinic with a history of dyspnea for five years. He had taken digitalis for about five months and had taken at least one digitalis tablet twice daily for a month prior to his visit. In spite of this edema developed and the patient had nausea and vomiting for a week before coming to the clinic. Physical examination revealed hypertension, enlargement of the heart to the left and irregular rhythm with a rate of 80. There were rales in the lung bases, hepatomegaly and edema of the ankles.

Requestioning uncovered the fact that the patient had procured some "Anademin" tablets (a preparation labeled as containing strophanthus $\frac{1}{1500}$ gr., squill $4\frac{3}{4}$ gr., canadian hemp $\frac{1}{64}$ gr. and elder flowers $\frac{1}{32}$ gr.) and had taken this medication on his own initiative "several" times a day in addition to his regular digitalis ration.

The patient was warned to discontinue the Anademin, and the dose of digitalis was reduced to 0.1 gm. daily. He was advised to remain under observation but he failed to do so.

Comment. This case illustrates the easily overlooked possibility of self-medication with preparations containing digitalis principles.

CASE VIII. A thirty-eight year old seven months' pregnant white female with toxemia

and edema was admitted to the hospital in active precipitate labor, which terminated in complete abortion on the way to the delivery room. She was given 6 "cat units" of digifolin® intramuscularly (probably because of the edema) and sent to the ward where an order was left for "digitalis 0.2 gram every six hours to *days* four." On the day after her admission the patient was thought to be acting strangely. The next day she was definitely irrational and refused all of her meals. On the third day she was quite confused and incoherent. She refused to eat; she had nausea and delusions; and she fell out of bed. The next day the order for digitalis was discovered on the patient's chart and the drug was discontinued. Apparently the order was meant to have been "digitalis 0.2 gram every six hours to *doses* four," but the patient had actually received a total of 6 cat units of digifolin® and 3 gm. of digitalis in ninety hours. An electrocardiogram showed second degree heart block with depression of the ST segment in lead I and inverted T waves in all leads. By the sixth hospital day the patient had become more rational but the pulse was irregular and she continued to have nausea and vomiting. On the ninth day an electrocardiogram showed P-R interval 0.17, normal sinus rhythm, T2 and 3 upright. The nausea and vomiting had disappeared.

Comment. The errors committed are obvious.

CLASSIFICATION OF MATERIAL

No doubt the data extracted from the analysis of only thirty-five cases are of strictly limited statistical value; yet the lessons to be derived from such a study are quite instructive and, it is believed, of some importance. The cases of poisoning being studied were classified according to the reason for which digitalis was employed. (Table I.) In determining why these patients received digitalis in the first place all available information was evaluated, including history, communications from the family physician, physical findings and subsequent course.

Seven patients of the thirty-five received digitalis because of inactive rheumatic heart disease with cardiac insufficiency. Four of the thirty-five patients were classified as having active rheumatic heart disease with mitral stenosis and insufficiency and cardiac failure. One of these had an associated aortic insufficiency thought to be due to syphilitic heart disease. There were four cases of arteriosclerotic heart disease with cardiac decompensation and five cases of hypertensive cardiovascular disease with decompensation. There were four cases of combined arteriosclerotic and hypertensive cardiovascular disease with cardiac insufficiency. One patient had syphilitic heart disease with aortic insufficiency and cardiac failure. It is obvious that the chief reason for the use of digitalis in the foregoing twenty-five cases was the existence of cardiac insufficiency.

TABLE I
REASONS FOR DIGITALIS THERAPY (THIRTY-FIVE CASES)

Condition	No. of Cases	Per cent
Cardiac insufficiency	25	71.4
Inactive rheumatic heart disease	7	20.0
Active rheumatic heart disease	4	11.4
Arteriosclerotic heart disease	4	11.4
Hypertensive cardiovascular disease	5	14.3
Arteriosclerotic and hypertensive	4	11.4
Syphilitic heart disease with aortic insufficiency	1	2.9
Rational explanation for digitalis lacking	10	28.6
Reasons for digitalis not apparent	3	8.6
Reasons for digitalis not rational	7	20.0

However, a rational explanation for digitalis therapy in the remaining ten cases was lacking. (Table I.) In three cases it could not be determined from the available information why digitalis was prescribed. One patient had questionable evidence of arteriosclerotic heart disease, questionable evidence of hypertensive cardiovascular disease and a very equivocal history of possible, but by no means probable, cardiac insufficiency. One patient with arteriosclerotic heart disease had no history of cardiac insufficiency but it is unknown whether he had auricular fibrillation prior to digitalis and it could not be determined whether or not the drug might have been given for this reason. Apparently the drug

was given originally to one patient because of dyspnea and weakness and, although anasarca subsequently developed in spite of digitalis therapy, no satisfactory etiologic diagnosis was made at necropsy. In seven cases the apparent reasons for using digitalis were not rational ones. There were two patients with toxemia of pregnancy but one had no objective evidence of cardiac insufficiency and the other terminated by abortion before digitalis was instituted. One patient with inactive rheumatic heart disease and mitral stenosis and insufficiency falls into this group because there had been no evidence of cardiac decompensation or auricular fibrillation. In one case digitalis was given because of a "pounding heart" although there was no evidence of cardiac failure; in another case it apparently was given because of a systolic murmur at the pulmonic area even though there was no evidence of cardiac insufficiency. Digitalis was given to two patients because of acute anxiety attacks which superficially simulated dyspnea although there was no objective evidence of cardiac decompensation. This group, then, is characterized by the fact that definite evidence of cardiac insufficiency was lacking prior to and at the time of instituting digitalis. Apparently, in these cases digitalis must have been given because of unsatisfactory or erroneous diagnoses or because of misinterpretation of the indications for digitalis therapy.

TABLE II
INCIDENCE OF CLINICAL SIGNS AND SYMPTOMS OF DIGITALIS POISONING (THIRTY-FIVE CASES)

Signs and Symptoms	No. of Cases	Per cent
Anorexia and nausea	25	71.4
Cardiac rhythm disturbances	25	71.4
Premature ventricular beats	20	57.1
with bigeminy	11 (55%)	
without bigeminy	9 (45%)	
Auricular fibrillation	14	40.0
fibrillating prior to digitalis	2 (14.3%)	
converted to fibrillation during digitalis	8 (57.1%)	
fibrillation prior to digitalis not known	4 (28.6%)	
with bradycardia	8 (57.1%)	
without bradycardia	6 (42.9%)	
Heart block clinically suspected	4	11.4
Vomiting	20	57.1
Visual disturbances	12	34.3
Diarrhea	7	20.0
Central nervous system symptoms	5	14.3
No clinical signs or symptoms	2	5.7

CLINICAL SYMPTOMS AND SIGNS OF POISONING

The thirty-five cases studied revealed some interesting facts about the symptoms and signs of digitalis intoxication. (Table II.) The gastrointestinal system may be affected by excessive doses of digitalis as evidenced by anorexia, salivation, nausea in waves, vomiting, diarrhea, abdominal discomfort and pain. Anorexia and nausea are usually the most common symptoms caused by digitalis; these occurred in twenty-five of the thirty-five cases. However, disturbances of cardiac rhythm occurred as frequently (twenty-five cases) in this series and were seen more commonly than vomiting (twenty cases) or diarrhea (seven cases). The most frequent type of rhythm disturbance encountered was premature ventricular beats which occurred in twenty of the twenty-five instances of arrhythmia. Of these twenty cases exhibiting premature beats, eleven were characterized by bigeminy. Auricular fibrillation was diagnosed in fourteen instances. Of these, conversion to auricular fibrillation during digitalis therapy occurred in eight while in two instances fibrillation previous to digitalis medication was known to exist; however, in four cases it could not be determined from the information available in the records whether or not the auricles were fibrillating prior to digitalis therapy. Of the fourteen cases with auricular fibrillation, the ventricular rate in eight was below 60 per minute, indicating a definite brady-

cardia. In the twenty-five rhythm disturbances observed, four cases of heart block could be suspected clinically from the physical findings.

The visual disturbances caused by digitalis include dimness of vision, inability to focus, scotomas, diplopia, blurring and yellow, green and white vision;[23] such symptoms occurred in twelve of the thirty-five cases of poisoning. The effects on the nervous system include headache, restlessness, irritability, disorientation, drowsiness, delusions, aphasia, ataxia, mania and stupor; in five of the thirty-five cases such nervous system symptoms were observed and were attributed to digitalis. Rare toxic effects of digitalis are eosinophilia, asthma and dermatidides, including erythematous, morbilliform, scarlatiniform, and exfoliative eruptions, urticaria and angioneurotic edema,[19] but no instance of these complications was observed in this series.

Two cases of the thirty-five failed to present reasonably suggestive clinical symptoms or signs of digitalis intoxication even though the poisoning was reaching a dangerous stage. It is important to realize that this can occur.

Although they are not always heeded, the more common signs and symptoms of digitalis poisoning (anorexia, nausea, vomiting and yellow vision) are certainly well known to the medical profession. However, many of the effects of digitalis are not widely appreciated (paroxysms of tachycardia, auricular fibrillation, mental disorientation, delusions and psychoses); yet they may occur, and not infrequently do, when the more well known manifestations are absent. Hence their importance is of more than academic interest. The appearance of any one sign or symptom of poisoning cannot be relied upon to mark the point of toxicity, for some signs and symptoms may occur with the slightest degree of intoxication in one case while the same signs and symptoms may never occur in another case with a severe degree of poisoning. Consequently, the physician must be familiar with the uncommon as well as the common signs and symptoms of digitalis poisoning and he must be alert to recognize them and to appreciate their significance.

ELECTROCARDIOGRAPHIC SIGNS OF POISONING

It is not within the scope of this discussion to describe in detail the electrocardiographic effects of digitalis, but it should be mentioned that these primarily consist of progressive changes in STT contour and shortening of the Q-T interval as therapeutic doses are increased. Prolongation of the P-R interval and increasing disturbances of the cardiac rhythm, conduction and irritability occur as toxic doses are reached. Digitalis effects may be manifested in the electrocardiogram by variations in the contour of the P waves, prolongation, distortion or alternation of the QRS complexes (rarely); by various degrees of sino-auricular block, auriculoventricular block and auriculoventricular dissociation; by auricular fibrillation, ectopic beats (auricular or ventricular in origin, often producing bigeminy or paroxysms of tachycardia), auricular standstill, ventricular tachycardia, chaotic heart action and ventricular fibrillation. Toxic signs and symptoms of involvement of the cardiac mechanism may simulate every known type of arrhythmia and electrocardiographic tracing.[17] These changes for the most part indicate that digitalis is depressing the conductive ability but enhancing the irritability of the myocardium.

Electrocardiograms were recorded in all but two of the cases in the group studied. The most common electrocardiographic effect seen in these thirty-three cases (Table III) were changes in the STT complex, sufficiently definite in twenty-five cases to be considered reasonably diagnostic of digitalis effects, while in six additional cases the changes in the STT complex were of such character as to be suggestive of and probably due to digitalis, but not definitely diagnostic. In the two remaining cases the characteristic changes of the STT complex were absent. In any event, the digitalis contour of the STT complex is not of itself

a sign of poisoning. Rather, it serves as a graphic warning that the drug is producing an increasing effect on the myocardium and that, as the effect continues to increase, toxicity is being approached.

TABLE III
INCIDENCE OF ELECTROCARDIOGRAPHIC EFFECTS OF DIGITALIS (THIRTY-THREE CASES)

Electrocardiographic Effect	No. of Cases	Per cent
STT complex changes	31	93.9
reasonably diagnostic	25 (80.6%)	
suggestive	6 (19.4%)	
Premature ventricular beats	14	42.4
with bigeminy	9 (64.3%)	
without bigeminy	5 (35.7%)	
Auricular fibrillation	9	27.3
fibrillating prior to digitalis	2 (22.2%)	
fibrillation prior to digitalis not known	4 (44.5%)	
converted to fibrillation during digitalis	3 (33.3%)	
with bradycardia	5 (55.5%)	
without bradycardia	4 (44.5%)	
First degree heart block	11	33.3
Second degree heart block	6	18.2
Sino-auricular block	2	6.1
Premature auricular beats	2	6.1
Complete heart block	2	6.1

The electrocardiogram revealed nine cases of auricular fibrillation, five of which had definite bradycardia. First degree heart block was detected in eleven of the thirty-three cases in which electrocardiograms were made, and six cases of second degree heart block were observed. Premature ventricular beats were present in the electrocardiograms of fourteen patients; these were characterized by bigeminy in nine cases. Two cases of sino-auricular bradycardia were observed electrocardiographically; one of these showed an alternately shifting pacemaker to the auriculoventricular node. Digitalis caused premature auricular beats in two cases detected by the electrocardiogram and one of these had runs of sino-auricular tachycardia. Complete heart block was observed in two cases.

While the electrocardiographic changes are frequently characteristic enough to be diagnostic of digitalis effects, there are borderline tracings wherein the degree of change due to digitalis and the degree due to myocardial disease may be extremely difficult to evaluate. Therefore, unless there is some urgent indication, digitalis should be avoided prior to electrocardiographic study of suspected myocardial disease because T wave changes may persist and obscure the picture for two to three weeks or longer after the drug has been discontinued. When a patient is receiving digitalis and doubt exists as to the cause of the changes seen, it usually is possible to discontinue the drug for a period sufficient to note the tendency toward disappearance or persistence of the abnormalities in the tracing, for unless these changes are due to the action of digitalis they are not necessarily contra-indications to the use of the drug.

Sino-auricular block is most commonly a result of digitalis intoxication and too much digitalis is said to be one of the commonest causes of fine auricular fibrillation.[17] Digitalis is a frequent cause of premature beats. Usually ventricular in origin, these beats are apt to be frequent and from multiple foci or in the form of bigeminy. When multiple premature ventricular beats with fixed coupling or several foci of origin appear in the electrocardiogram of the patient receiving digitalis, they should serve as a distinct warning to withhold the drug, even if the STT complex variation characteristic of digitalis effects is absent. When disorders of cardiac mechanism are found to be due to digitalis, a high percentage of the lethal dose (between 50 and 90 per cent) has probably been given.[15] In cats the STT complex changes appear after 25 per cent of the lethal dose of the drug has been given, the P-R interval is increased after 53 per cent and ectopic beats appear after 72 per cent of the lethal dose has been administered.[4] On the other hand, not every instance of digitalis intoxication will show the characteristic STT complex change and it cannot be relied upon as an unerring

indication of excessive digitalis.[17] Therefore, any one of these changes should serve as a warning that lethal doses are being approached and one must proceed with extreme caution.

CAUSES OF POISONING

In reviewing the thirty-five cases (Table IV) it was found that five cases of poisoning were, at least in part, due to the patients themselves because of self-medication or unauthorized adjustment of the dose of digitalis. No case in this series was believed to be intentionally self-inflicted although such a purpose often can be easily accomplished and could be most difficult to evaluate. On the other hand, the physician was directly responsible for three cases because of mistakes in prescribing, ordering or supervising the administration of the drug. Furthermore, in ten of the thirty-five cases, as has been pointed out, we could not rationally explain the use of digitalis in the first place. Moreover, poisoning occurred in twenty-six cases because definite clinical signs and symptoms of digitalis intoxication were not appreciated or were ignored. Only two patients exhibited no definite clinical warning signs or symptoms of poisoning in spite of its presence to a dangerous degree; but, even so, the drug definitely should not have been used in at least one of these cases.

Considering the obvious confusion that apparently existed in regard to the indications for digitalis, it is hardly surprising that the warning signs and symptoms of poisoning were not recognized and heeded. However, it is difficult to determine specifically why they were ignored. In some cases, perhaps, observation was not close enough but in others this certainly was not true. The largest single factor seemed to be that the signs and symptoms, although observed, were not recognized as indicating digitalis poisoning or, if they were recognized, they were erroneously interpreted as being of little or no importance.

In at least eighteen of the cases the patients could reasonably be presumed to have been on doses of digitalis significantly greater than the accepted average dose, which should in itself have brought attention to the necessity for caution and close observation. Of the twenty-five patients with cardiac insufficiency, seventeen were poisoned by the drug after having achieved a definite therapeutic response. Again, this should have warned the physician that the dosage level was a potent one.

TABLE IV
INCIDENCE OF CAUSES OF POISONING (THIRTY-FIVE CASES)

Cause	No. of Cases	Per cent
Due to patient (at least partially)	5	14.3
Errors in prescribing	3	8.6
Rational explanation for digitalis lacking	10	28.6
Reasons for digitalis not apparent	3	8.6
Reasons for digitalis not rational	7	20.0
Signs and symptoms of poisoning ignored	26	74.3
Excessive dependence upon digitalis and neglect of other therapeutic measures	8	22.9
Poisoning obscured by concurrent disease	4	11.4

It is true that in some cases (eight of the twenty-five patients with cardiac insufficiency) adequate therapeutic response was not achieved in spite of digitalis in such amounts that poisoning occurred. The principal error committed in these instances was that too much reliance was placed on digitalis therapy to the neglect of the other aspects of treatment for congestive failure. Of primary importance in this respect is the control of the patient's activities, in order to reduce the cardiac load as much as possible, conserving the integrity of the heart. Digitalis given without control of activities is not reasonable, for when it is used only for the purpose of increasing the patient's exercise tolerance, the drug loses its analogy to a crutch and becomes more comparable to a whip.

There were four cases of the thirty-five in which it was believed that the signs and symptoms of digitalis intoxication were confused or obscured by concurrent diseases so that the diagnosis was not immediately evident. As an example, one patient, who

was receiving digitalis to maintain compensation in the face of arteriosclerotic heart disease with a history of failure, was found to have x-ray evidence of carcinoma of the stomach. His symptoms of anorexia, nausea, vomiting and abdominal pain were attributed to the gastric carcinoma until cardiographic signs of digitalis intoxication were discovered. Appropriate reduction of the digitalis ration temporarily resulted in complete disappearance of his troublesome symptoms. This illustrates that any patient receiving digitalis must be suspiciously evaluated with the possibility of poisoning in mind when that patient's course is not satisfactory. In any case in which confusion exists in regard to the degree of digitalization the safest course to follow is to withhold the drug for a few days, relying on other means of maintaining cardiac compensation, until observation of the effect of withdrawal can be made. Undoubtedly, fewer untoward results will occur with such a program than if digitalis were continued when there is uncertainty about the degree of digitalization.

COMMENTS

The proper administration of digitalis is essential to avoid poisoning with the drug. It is important to realize that the amount of digitalis required is influenced by a number of factors including body weight, status of the myocardium, preparation used, rate of absorption, speed of mobilization and excretion and amount of edematous fluid. In general, ambulatory patients have a fairly wide margin between the doses producing therapeutic and toxic effect whereas in severe cases of heart disease this margin is apt to become narrow. In addition, the rate of excretion of digitalis will vary with the amount in the body and the amount mobilized. The physician should be aware that the administration of mercurial diuretics to certain patients with congestive failure who have received considerable digitalis may be followed by rapidly increasing digitalis effects or intoxication, perhaps due to the mobilization of digitalis-containing fluid from the tissues and serous cavities.[20] Moreover, the requirements for the drug change in the same patient as the disease progresses or regresses, and there is a wide variation from patient to patient in the dosage level found most effective, indicating various degrees of individual tolerance to the drug. The recognition of this variation in susceptibility is a primary prerequisite for the human assay of the drug by electrocardiographic methods[7] since some individuals are able to distinguish between a 25 per cent dosage difference electrocardiographically while others cannot do so. It must be remembered that a digitalizing dose of ± 25 per cent of the mean digitalizing dose includes only 70 per cent of patients.[7]

Digitalis is retained as successive doses are given; this cumulative action is a characteristic which will lead to poisoning if it is given in excessive quantity, too rapidly or over too long a period. Considerable variation exists in the percentage absorption and duration of action of the various digitalis preparations. While digitoxin is completely absorbed, only about 20 per cent of the digitalis leaf and 10 per cent of Lanatoside C is absorbed.[7] On the other hand, the maximum effect of a digitalizing dose of digitoxin, given orally or intravenously, occurs between two and nine hours after administration, while a digitalizing dose of Lanatoside C, given intravenously, has its maximum effects between one and a half and two and a half hours. Furthermore, the effects of a digitalizing dose of Lanatoside C disappear within two and a half to six days whereas the effects of a digitalizing dose of digitoxin may require from two to three weeks to disappear.[24] Thus cumulation of the drug depends on its administration in relation to these factors (percentage absorbability, time of maximum effect and duration of action) characterizing the various preparations.

In general, as far as the various glycosides are concerned the therapeutic ratios are about the same. That is to say, when the minimum maintenance dose is doubled,

toxicity occurs in about the same percentage of cases (62 to 66 per cent) regardless of the glycoside being used.[25] Furthermore, if maintenance with digitalis leaf is not satisfactory and toxicity occurs at the next dosage level, the administration of a glycoside results in the same response if comparable doses are used. In addition, the toxic effects of the cardiac glycosides are generally similar to those of the crude drug, but there are two differences worth noting. Diarrhea is more unusual and, while the visual disturbances are just as common, yellow vision and green vision are not as frequently encountered when the purified glycosides are used.[25] A non-toxic digitalis preparation, if effective at all, does not exist.

The dangers involved in the indiscriminate use of various preparations of digitalis are well known for Gold[7] has shown by the human assay method that one specimen of digitalis preparation labeled 1 U. S. P. unit may produce three times as much effect as another. Furthermore, one preparation of the purified glycosides may produce ten times as much effect as another with the same U. S. P. unitage. Others[2,11,24] have pointed out the confusion which has resulted because of changes in the standardization of the U. S. P. units, and pleas have been made for the individual physician to select one acceptable preparation and thoroughly familiarize himself with the use of the one product rather than attempting to gain experience with various preparations unfamiliar to him.

The route of choice for the administration of digitalis is the oral one, for fatalities rarely occur when the drug is given by mouth.[10] Overdigitalization by the intravenous route can be accomplished too easily, and it may be so rapid that irreparable damage may occur before "any of the signals of forebearance" appear. Tandowsky et al.[18] electrocardiographically illustrate such a case in which ouabain was given intravenously before a history of digitalis maintenance was elicited, with the result that ventricular fibrillation and death ensued within sixty seconds. There is a distinct hazard in using intravenous digitalis preparations, particularly when one is not certain that the drug has not been used in other forms within two to three weeks. Moreover, the symptoms of failure initially bring most patients to their physicians long before the decompensation is severe enough to warrant rapid digitalization. In those rare instances in which it is necessary to give a digitalis preparation parenterally because quick action is desired, ouabain intravenously is the preparation of choice.[26]

Since digitoxin is absorbed completely from the gastrointestinal tract, the intravenous dosage of this preparation corresponds with its oral dosage; and because of this it has recently found considerable favor in the profession. Nonetheless, this does not decrease the considerable variation in the amount of the drug necessary to produce desired effects in various individuals; hence it is safer and more logical to give less than the digitalizing dose and to achieve digitalization gradually.[26] Even though the intravenous dose may be the same as the oral, the optimum dosage still must be determined individually from the response of the patient and no predetermined dosage schedule can fulfill this responsibility.[27]

The optimum dose of digitalis should not be defined as the dose which falls just short of toxicity for that implies that toxic doses must be given in order to determine the optimal dose. In most patients the optimal dose is well below the toxic dose, but this therapeutic ratio may vary considerably, depending largely on the state of the myocardium. It is more sensible to define the optimum dose as the minimum dose required to produce the desired effect. This dose must be determined individually for each patient since it is potentially different in every case.

The physician should watch for therapeutic effects in order to adjust the proper dosage of digitalis rather than to rely on the appearance of toxic symptoms. The relief of congestive failure is the most reliable

indication that effective doses are being employed. Abatement of a rapid ventricular rate, especially when associated with auricular fibrillation, is another valuable sign that an effective dose has been reached. Extremely useful and clinically accurate gauges for following the response of decompensation or its degree of recurrence are frequently overlooked, and these are the relatively simple expedients of observing weight changes and urinary outputs.

On the other hand, it is worthy of emphasis that certain patients with congestive failure can derive only strictly limited benefit from digitalis because of extensive myocardial damage, and forcing the drug to toxic effects cannot be expected to bring relief in direct proportion to the amount of the drug administered. For this reason the degree of improvement with digitalis is of some prognostic value. Nonetheless, one must not overlook the fact that other measures (activity restriction, salt elimination and mercurial diuretics) often will assist in controlling congestive failure when the benefit from digitalis alone is limited.

SUMMARY AND CONCLUSIONS

1. The principal pharmacologic effects of digitalis are an increase in the force of cardiac contraction, a slowing of conduction through the auriculoventricular node and an increase in myocardial irritability.

2. The indications for and contraindications to the use of digitalis are based logically on the pharmacologic properties of the drug. Yet misuse of digitalis occurs too frequently.

3. A total of thirty-five cases of poisoning by digitalis and related drugs has been collected from records and recent experience. Reports of eight cases are presented and commented upon. The material collected has been analyzed from the standpoint of the reasons for digitalis therapy, the incidence of the signs and symptoms of poisoning with digitalis and the causative factors leading to poisoning.

4. Information pertinent to the proper use of digitalis has been emphasized and discussed.

5. The physician should acquire a sound, practical knowledge of the indications for and the contraindications to the use of digitalis and its allies. He must be familiar with the dosage, action and effects (usual and unusual) of the preparation selected and he must employ discrimination, judgment and caution in the use of a valuable but potentially dangerous agent.

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Seminars on Cancer Research

Aspects of Approaches in Experimental Cancer Chemotherapy*

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Although earlier investigators and practitioners in cancer chemotherapy may have held hopes for success as high as those today, the experimentalists currently seeking chemical agents effective in the treatment of neoplasia have a sounder basis for their optimism. The constant accumulation of fundamental knowledge in biology, the development of new equipment and technics for study, particularly the use of isotopes for developing information on metabolism, and the striking achievements in the control of bacterial cells by antibiotics give substantial reasons for expecting a fruitful outcome of cancer research. The optimism aroused by the great strides in antibacterial therapy is tempered, however, by the sober fact of the greater magnitude of the task confronting the cancer specialist who must deal with (1) the larger variations between bacteria and normal mammalian cells than between the latter and mammalian cancer cells, (2) the more time-consuming and costly tests still required in anticancer studies and (3) the lack of early defense by the host against renewed growth of a few cancer cells surviving drastic chemotherapeutic measures in contrast to the ability of the animal to complete the elimination of bacteria after immunologic or other chemotherapeutic intervention. Cancer research, in its need for fundamental information, presents a challenge to all branches of modern biologic research. The need for this great effort is apparent when the few, inadequate agents for clinical use are considered as in the next article of this series[1] and in recent reviews.[2–5]

The present paper will not attempt to review the numerous studies in the experimental chemotherapy of cancer but will seek to discuss various considerations involved in such studies. Different aspects of approaches in experimental cancer chemotherapy will be illustrated by examples from a number of studies and in particular from a program with which we have been identified.[6]

Regardless of how it may be described or how many steps there may be in the study, the goal of any experimental cancer chemotherapy program is the discovery of materials which will affect tumor tissue adversely in a selective manner. To obtain this differential effect there is required in the cancer cell a deviation from the normal sufficient for it to be subject to attack as a parasite in the classic concept of Ehrlich.[7] The cancer cell has been likened to the mutant bacterium which has survived unfavorable conditions and is recognized by its characteristic metabolic or morphologic differences from the normal.[8] The uncontrolled growth of cancer tissue and its morphology mark it as different from the tissue of origin, whether that change has been due to the introduction of a virus[9] or to modification of a nucleoprotein or other important cellular constituent by physical agents or by carcino-

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gens.[10] Regardless of the cause of cancer, this abnormal cell would appear to pose a problem resembling in many respects that faced by those seeking non-immunologic agents to combat virus infections. Metabolic differences between neoplastic and normal tissues have been sought extensively[11] but those found have not been sufficient to provide adequately for selection of candidate chemotherapeutic agents. An observation of interest[12] is the increased ability of hepatoma cells arising from dietary butter yellow to metabolize this compound as compared with normal hepatic cells. Such a demonstrable difference and the availability of a few compounds showing selective though inadequate effects give encouragement for conduct of major programs seeking more compounds of greater effectiveness.

Many of the compounds tested for antitumor activity have been tried empirically because of the deficient knowledge of differences between normal and cancer cells. Even if this were the sole basis for selection of test materials, it could be justified by the urgency of the problem and in the light of the numerous empirical developments in the history of medicine. It is, however, intellectually more satisfying to pursue a program in which compounds are chosen on a more rational basis. This is being done in a number of instances by testing compounds which are: (1) related in structure to the few compounds already known to exhibit some anti-tumor effects;[13,14] (2) possible antimetabolites[15,16] and more specifically substances which might interfere with nucleoprotein metabolism;[17–19] (3) materials demonstrated to have some striking effect in a biologic system.[20]

The history of science reveals a number of instances in which advances were made possible only when certain tools or technics were provided. Experimental cancer chemotherapy owes much to Loeb[21] and Morau[22] who demonstrated the practicability of transplantation of tumors in laboratory animals. This removed the limitations imposed by lack of an adequate supply of uniform tumors when those occurring spontaneously constituted the sole source. Since then numerous investigators, using nearly as many different technics and various spontaneous and induced transplantable tumors, have sought in isolated experiments or extensive programs to find materials harmful to tumors.[6,20,23,24] The technics have differed in the usual variables encountered in any chemotherapy studies, such as the species of host and the route, time of initiation, frequency and level of injections. To the usual problems of rates of absorption, excretion and detoxification or destruction are added such questions as the best tumor to be employed and whether the results in experimental animals can be translated to a human tumor, a difficult matter because of the paucity of compounds effective in human tumors which could be used as yardsticks to measure the correspondence of results in mouse and man. With compounds of limited clinical effectiveness there are indications that mouse tumors offer useful screening devices when intelligently employed. Actually, it will be seen that there may be more difficulty in transferring the results from one type of experimental tumor to another than from a mouse tumor to its human prototype.

Some of the points discussed in general terms may be clarified or emphasized by specific examples given in the following discussion of the chemotherapy studies being conducted in our laboratories.[6,*] This program involves a multiple approach in which a multiplicity of substances is being tested against a number of tumors under various conditions. The organization of the program is shown in Figure 1. The common goal of the different research activities is the discovery of substances which will selectively show adverse effects upon tumor tissue. To this end synthetic chemicals,

* This cooperative program is being carried out by a group of investigators responsible for its different phases as follows: pharmacology, Dr. F. S. Philips; tissue culture, Dr. John J. Biesele; egg culture, Dr. David A. Karnofsky; leukemia, Dr. Joseph H. Burchenal; solid tumor screening, Dr. Sonja Buckley; solid tumor spectrum, Dr. Kanematsu Sugiura; virus studies, Dr. Alice E. Moore; and antibiotics, Dr. H. Christine Reilly.

biologic entities such as viruses, and other materials of natural origin including extracts and compounds from the higher plants and metabolic products of bacteria and fungi, are being screened against Sarcoma 180[25] and in leukemic mice.[14]

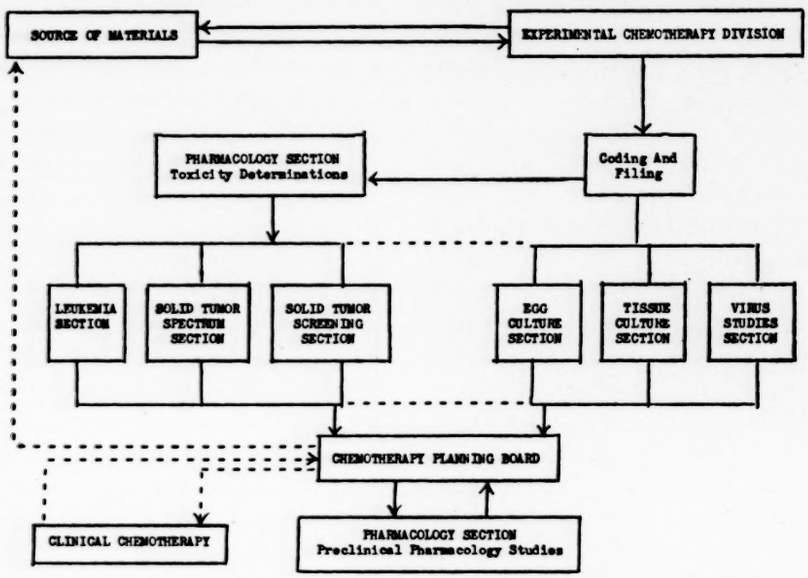


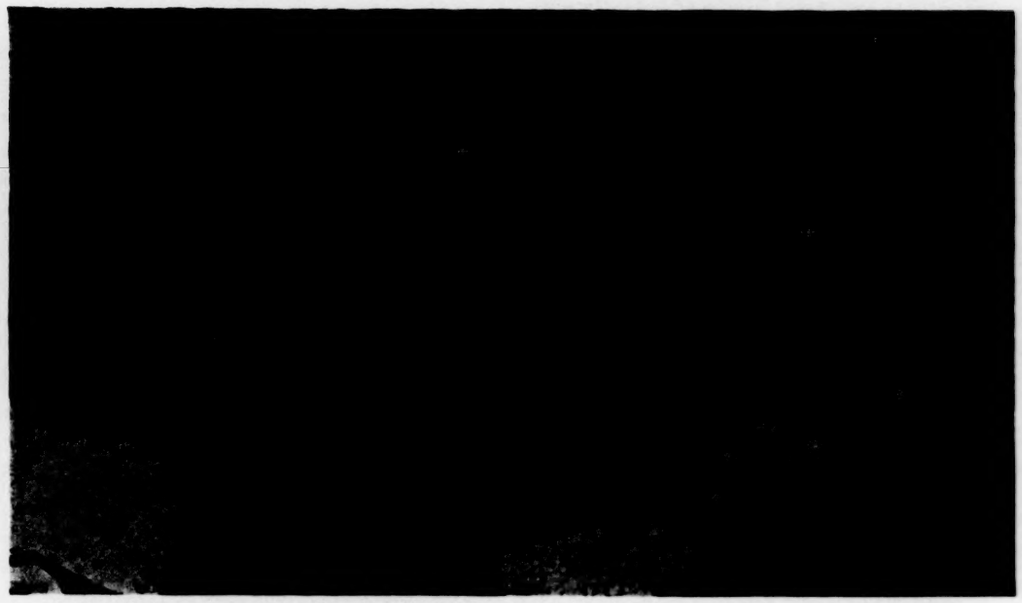
FIG. 1.

Supplementary studies in tissue culture[26] in the chick embryo[13] and in a spectrum of rat and mouse tumors[27] also are conducted. Each test possesses certain advantages as well as limitations which must be taken into consideration.

In vitro tests have been used by a number of investigators.[25,28,29] This has been prompted by a desire to gain additional information under the conditions of the test or to find a quick, simple method for screening candidate materials. Some of the tests[25,30] have involved incubation of fragments of a tumor in solutions of the test materials under conditions of pH, tonicity, temperature and incubation time normally well tolerated by the tumor. After incubation the effect of the material on the tumor fragments is assayed by determination of their transplantability to susceptible hosts. While this has revealed information about lack of direct action by certain substances, there may be pitfalls in the interpretation of positive effects, e. g., NH_4 ion has rendered tumor fragments non-viable with the effect initially attributed to another substance under test. An *in vitro* test which would quickly and easily give results significant for *in vivo* studies would be a powerful aid in speeding studies of antitumor agents.

Tissue culture is an *in vitro* technic in which normal and neoplastic cells may be exposed to an agent under conditions more favorable for growth and maintenance of cells. It has the advantage of permitting experiments with substances available only in limited amounts and provides information on materials in the absence of modifying effects of the intact host, e. g., excretion, detoxification, destruction or unusual distribution in the body. Though tissue culture offers the possibility of finding substances with a selective action on tumor tissue, those materials may for a number of obvious reasons be ineffective *in vivo*. Modification which might render the substances effective *in vivo* can then be considered.

Roller tube cultures of the type employed by Gey[31,32] have been useful. Explants of mouse tumor and normal embryonic tissue are fixed by chicken plasma clots on the inner wall of test tubes and nutrient solution added. The tubes are incubated twenty-four hours at 37°C. in a

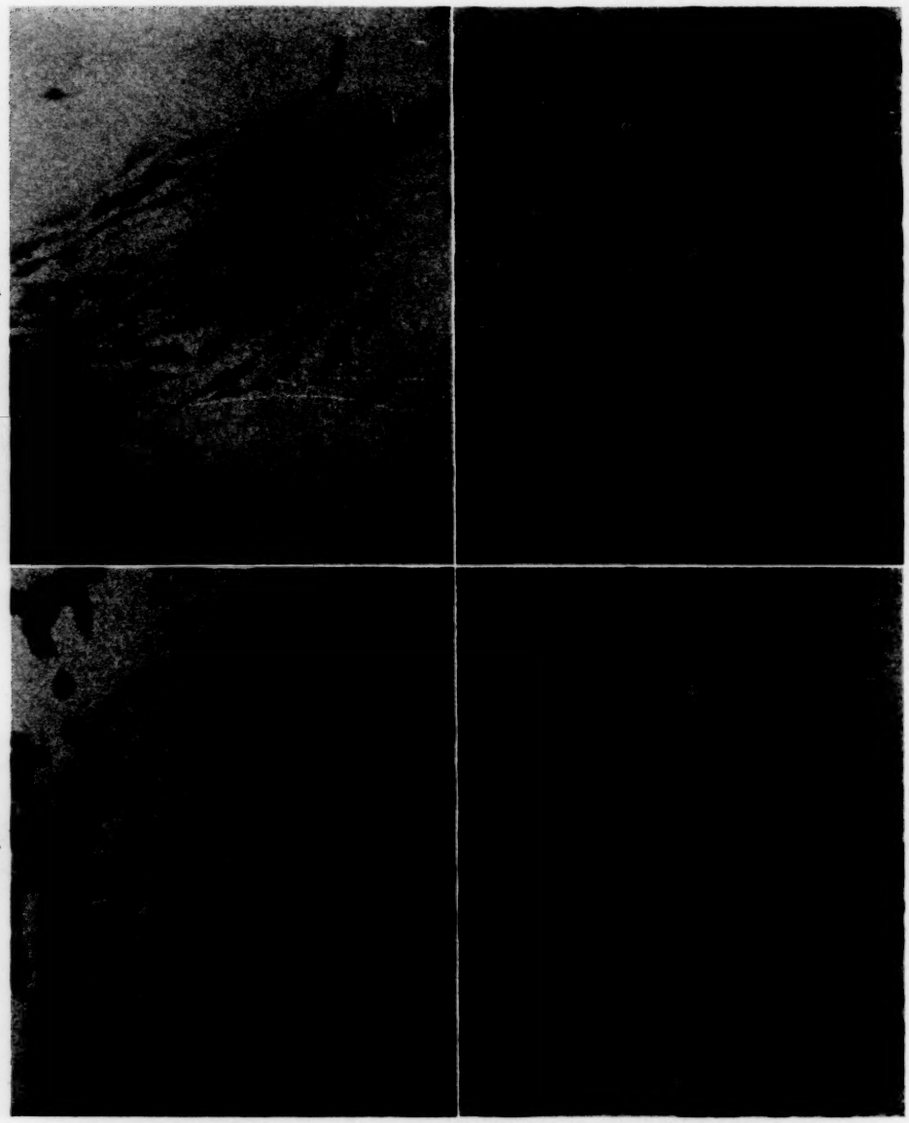


A B

FIG. 2. Damage by podophyllin to mouse sarcoma MA 387 in tissue culture; A, normal culture; B, a culture after exposure to 12γ podophyllin.

rotating drum after which the tissue implants are evaluated for their condition and amount of outgrowth. During the subsequent twenty-four-hour period the tubes are incubated with test material added to the nutrient solution in an amount previously determined to be tolerated by the normal tissue. After the twenty-four-hour exposure to the test material the amount of growth and condition of the normal and tumor implants are compared. In some instances interesting differential effects have been observed.[26] Figure 2 shows the damage caused to mouse Sarcoma MA 387 by podophyllin at a concentration tolerated by mouse embryo epithelium in the same tube. The differential effect of this material was found independently by a different technic in another laboratory.[33] A second example is 2,6-diaminopurine which has shown a differential effect on several mouse tumors compared with embryonic skin.[16] As illustrated in Figures 3 and 4, mouse Sarcoma T241 has been damaged at levels of the compound non-toxic to normal tissue. This compound has been shown to inhibit the growth of bacteria,[34] to decrease the production of vaccinia virus in tissue culture[35] and to effect adversely leukemia in mice.[18] It has been ineffective against nearly all of the solid tumors tested. (Table VI.) While tissue culture has certain inherent limitations, it will be a technic of increasing interest and value in view of the developments of Earle[36] on methods for producing cultures from single cells and cultivating increased amounts of cells, the elaboration of more precisely defined nutrient media[37–39] and studies on better methods of evaluation of damage by test materials.[40]

Studies of tumors growing on chorioallantoic membrane were initiated by Murphy.[41] The procedure developed by Karnofsky[13] for finding materials active against tumors on the membrane is as follows: (1) Tumors are placed on chorioallantoic membrane of eight-day egg. The eggs are first candled to determine viability and to locate a good supply of blood vessels for placement of the tumor fragment. The hole cut in the shell for introduction of the piece of tumor is sealed with a washer and glass cover slip to facilitate subsequent observation of tumor growth. (2) When the egg is twelve days old, the maximum nonlethal dose of chemical is injected into the yolk-sac. (3) Five days later the seventeen-day old egg is opened and the tumor re-



3A 3B

4A 4B

FIG. 3. Mouse embryo epithelium in tissue culture; A, control culture; B, tissue exposed to 2,6-diaminopurine 75γ/ml. Note similarity in condition of the cultures.
FIG. 4. Mouse sarcoma T 241 in tissue culture; A, untreated culture; B, culture exposed to 2,6-diaminopurine 75γ ml. Note extensive damage from the 2,6-diaminopurine.

moved for bio-assay in mice and for histologic evaluation of damage. Figure 5 shows a tumor after ten days of growth on the membrane.

This procedure offers an opportunity to test materials which must pass through a biologic system while coming into contact with the tumor. The chick embryo presents a closed system with characteristics peculiar to the species and its early stage of development. Substances damaging the tumor on the membrane without harming the embryo cause an effect in which the observed differential is between abnormal mammalian tissue and normal chick embryo tissue under the specific conditions of the test. Only nitrogen mustards and related compounds have severely damaged mouse Sarcoma 180 on the membrane at levels permitting the chick to hatch. Of equal interest are some striking abnormalities in the embryo produced by a few compounds.

Some of these are presented in Table I; the data were obtained in younger embryos. In addition there are listed the chemotherapeutic doses of representative materials and any tumor damage revealed by the standard technic described above. The compounds causing bizarre effects or more or less specific damage to organs or systems appear worthy of further study. The materials may be useful tools in fundamental studies of embryonic development and also provide agents for treatment of tumors arising from tissue of the same type affected in the chick embryo.

Studies in pharmacology are an essential aspect of the chemotherapy program. (Fig. 1.) This section of the program has a dual role. First, toxicity data are required to guide administration of the test materials in experimental cancer studies in animals with leukemia or solid tumors. In the second instance, those materials of sufficient promise to be considered for clinical trial demand more extensive, detailed pharmacologic investigation. Studies of this nature indicate possible adverse effects upon normal tissues and define limitations of clinical dosage. In addition, fundamental information upon mechanisms of the observed tumor effects may be provided for some compounds.[42–44]

The toxicity determinations provide data permitting selection of the maximum dose tolerated on repeated injection. This has been determined routinely for each compound available in adequate amounts prior to any tumor studies in animals.[45] The procedure adopted in the toxicity studies has been one requiring a minimum of mice consistent with the provision of sufficiently reliable data. Briefly described, the compound is injected intraperitoneally on each of five consecutive days into three mice at each dose level. Five or six groups of mice are used; each group receives a different dose level with the levels representing successive decreases of 50 per cent. Typical data are presented in Table II.

A procedure used for determining compounds effective against mouse leukemia

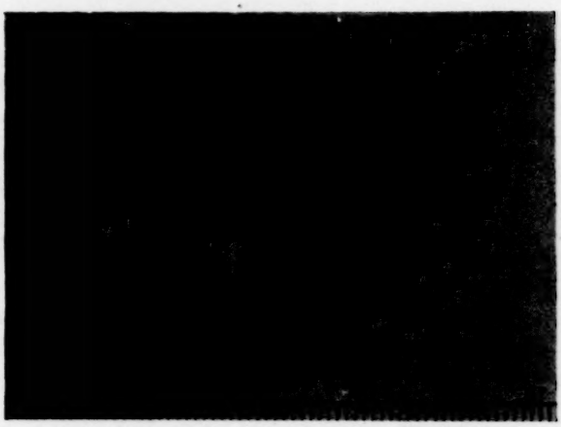


FIG. 5. Mouse tumor on the chorioallantoic membrane of the chick embryo. The egg has been opened to show the tumor which had grown ten days on the membrane.

is a modification[14] of one developed by Furth.[46] Leukemia occurs spontaneously in an inbred strain (Akm) of mice, but for chemotherapy studies the mice are given leukemia by intraperitoneal injection of suspensions containing 1,000,000 cells from the minced spleen of a mouse acutely ill with leukemia. The Akm mice so injected all die with little variation within a definite time period whereas other mice without this genetic background usually are unaffected, another example of the role of genetics in cancer.[47] The time of death of the Akm mice from leukemia is sufficiently definite that increases in survival time of treated animals can be used as a measure of the effectiveness of the treatment. This routinely consists of a course of intraperitoneal injections of the maximum tolerated dose three times weekly for a total of ten injections. Administration of the substance is started two days after injection of the leukemic cells. Increases in survival time below a selected minimum are discounted for they may be due to general toxicity or some non-specific cause. Some materials have caused a 100 per cent or greater prolongation in survival of treated animals beyond that of the untreated controls, e. g., the nitrogen mustards against chloroleukemia Ak 1394 and antifolics against lymphoid leukemia Ak4. The approximate results obtained with a few compounds are compared for four strains of leukemia, Ak

TABLE I

TOXICITY OF SEVERAL AGENTS TO THE FOUR-DAY OLD CHICK EMBRYO AND THEIR EFFECT ON EMBRYONIC DEVELOPMENT; THE EFFECT OF SOME OF THESE AGENTS ON SARCOMA 180 GROWING ON THE CHORIOALLANTOIC MEMBRANE

Compound	Embryologic Studies Four-day Embryo		Chemotherapy Studies Twelve-day Embryo		Reference Nos.
	Maximum Tolerated Dose (mg./egg)	Developmental Anomalies	Chemotherapy Dose (mg./egg)	Effect on Sarcoma 180	
Podophyllotoxin	.....	.....	0.0015	None	80
Colchicine	0.003	None	0.015	None	80
4-Amino pteroylglutamic acid	0.003	Marked growth inhibition; evisceration; parrot beak	0.10	Tumor degeneration but a few viable cells remain	16, 81
Methyl bis (β-chlorethyl) amine HCl	0.015	Occasional growth inhibition; edema	0.2	Severe tumor damage, cell viability destroyed	13
2,6-Diaminopurine lactate	1.0	Anemia, edema, abnormal lower beak	2.0	None	81
Thallium sulfate	1.0	Achondroplasia	2.0	None	80
Methoxypyridoxine	0.17	None	5.0	None	79
Desoxypyridoxine	4.0	Slight stunting and occasional developmental abnormalities	5.0	None	79
Streptomycin	.....	.....	25.0	None	80
Benzene	.....	.....	30.0	None	80
Urethane	200.0	Stunting, edema, occasional evisceration	100.0	None	80

TABLE II

TOXICITY DATA FOR 2,4,6,7-TETRAHYDROXYPTERIDINE IN MICE*

Dose (mg./kg.)	Mortality†	Day of Death	Weight Response
500	3/3	1, 1, 2	.....
250	3/3	1, 1, 2	.....
125	3/3	2, 2, 2	.....
62.5	3/3	1, 2, 2	.....
31.3	3/3	2, 2, 2	.....
15.6	1/3	2, –, –	.....
7.8	0/3	–, –, –	+0.4 gm.

* The mice were injected intraperitoneally once each day for five days with 0.5 cc. of the gum acacia-saline suspension of the compound at the indicated dose. The time of death of each mouse and the average weight change one week after starting the injections were recorded.

† Numerator refers to number of mice dying; denominator refers to the number in the test group.

TABLE III

APPROXIMATE EFFECTS OF COMPOUNDS AGAINST DIFFERENT STRAINS OF MOUSE LEUKEMIA

Compound	Representative Increases in Survival Time in Different Strains of Leukemia—Per cent Prolongation in Survival Time of Treated Animals beyond that of Untreated Controls*			
	Ak 1394	Ak 4	F T_{13} Line 15	F T_8 Line 291
Methyl bis(β-chlorethyl)amine HCl	100	40	.....	.....
4-Amino pteroylglutamic acid	0	50	.....	.....
4-Amino-N^{10}-pteroylglutamic acid	20	150	.....	.....
Urethane	100	20	100–200	25–50
Urethane of 2,7-diaminofluorene	0	0	.....	.....
Potassium arsenite	60	0	300–350	10–40
Benzene	100	0	40–60	0–25

* These percentages are only rough approximations to show relative susceptibilities of different strains. The data were derived for Ak 1394 and Ak 4 from Burchenal[14,18,58] and for the F strains from Kirschbaum.[82]

1394, Ak4 and two F substrains in Table III. These are representative of many compounds that are not uniform in effectiveness against different strains of leukemia. The studies in leukemia offer interesting extremes of what may be found in studies of a series of related compounds. Among the nitrogen mustards nearly all of those possessing two reactive β-halogenated ethyl groups are active against selected strains of leukemia. Compounds with a single β-chloroethyl amine group are inactive.[14] On the other hand, the carbamates, a group of compounds studied extensively in leukemia[5,14,48] and at least one other experimental tumor,[49] have been without significant effect thus far with the single exception of urethane (ethyl carbamate). Studies made since the activity of urethane in human leukemia was first observed[50] have, to the present time, emphasized its unique effectiveness against leukemia and Sarcoma 180. This is in contrast to other effects exhibited in common by some carbamates, e. g., hypnosis,[51] carcinogenesis,[52] leucopenic action,[53] mitotic inhibition[54] and antitumor activity in tests with the Walker carcino-sarcoma 256.[55]

The mouse leukemia studies have led to selection of certain compounds for clinical trial and thus far there has appeared to be a good parallel in the observations on mouse and man. The antifolics, nitrogen mustards and urethane have shown definite but inadequate action on leukemias in mice. These agents have found use in certain types of leukemia in man but the effects have been temporary.[1–4,56,57] Even differences in the effectiveness of the drugs in man have been reflected in the results on various types of leukemia in mice.[58] A comparison of the results adds support to the validity of the method as a tool for selecting compounds for clinical trial.

The selection of a tumor and the conditions of the chemotherapy screening program in which it is employed can be made so severe that only those materials of outstanding merit will survive challenge of the test. At the other extreme the choice of tumor and test procedure may be such as to permit frequent misguided announcements of "cancer cures." It has appeared desirable to select a tumor of moderate susceptibility to a few agents, such as x-ray and the nitrogen mustards, and to test it under conditions permitting the uncovering of new leads for synthesis of more effective compounds. Sarcoma 180 is considered to be of average sensitivity[59] and has been selected as the solid tumor for primary screening in the program under discussion, also because it is routinely transplanted with nearly 100 per cent success, it grows rapidly and the regression rate is low, negligible in a short-term test. Moreover, an inbred strain of mice is not required, an added practical advantage outweighing theoretic objections.

Various methods of treatment and evaluation of adverse tumor effects have been utilized. Included among the latter are gross and histologic evaluation of tumor damage after administration of a single large dose of test material to the host;[20] determination of the comparative weights of tumors in treated and untreated animals;[60] comparison of growth rates by measurements of diameters of tumors during a treatment and pretreatment period;[61] measuring diameters of tumors after a selected period of therapy or of inhibition treatment;[6,60] and prolongation in survival times of treated animals as mentioned in the discussion of the leukemia studies.[62]

A sensitive technic favoring demonstration of activity of compounds *in vivo* has been adopted in the study with Sarcoma 180.[6,25] It is understood that some false positive results may be obtained which can easily be eliminated by subsequent, more rigorous tests in Sarcoma 180 and by supplementary experiments in the spectrum of tumors. Under conditions of the routine test the unusually effective compound will be inescapable.

The routine screening test in Sarcoma 180 is outlined as follows: (1) Weigh mice individually. (2) Transplant tumor fragment subcutaneously into axillary region by tro-

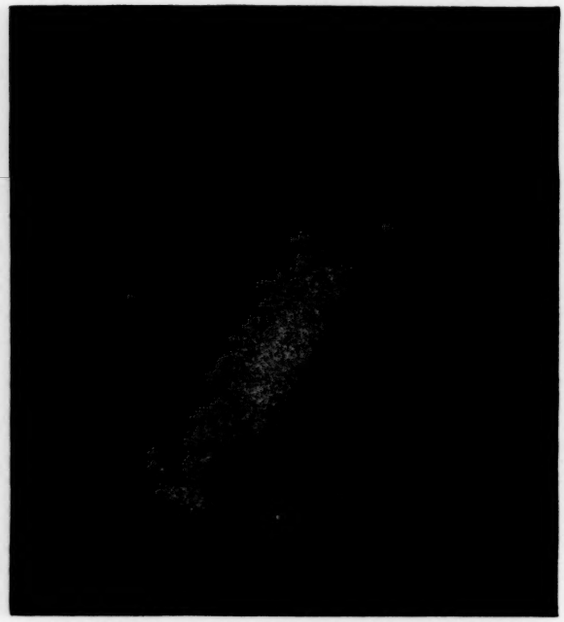


FIG. 6. Implantation of tumor fragment subcutaneously by trocar.

car, which is inserted initially into the inguinal region. (Fig. 6.) (3) Twenty-four hours later start intraperitoneal injections of the test material in the maximum tolerated dose into each of five mice. (4) Continue injections twice a day for seven days. (5) On the eighth day weigh each mouse and measure each tumor in two diameters with calipers.*

When compounds are found to be either markedly or moderately inhibitory in the first test, they are studied in repetitive tests to determine the reproducibility of the findings. Materials more or less consistently showing inhibition of Sarcoma 180 are candidates for similar study in a spectrum of tumors. More rigorous therapy trials against Sarcoma 180 are also employed. In these experiments the tumor is allowed to grow for more than one day before therapy is started even though it is uncertain what age of a transplantable mouse tumor is comparable to the majority of tumors presented as clinical problems.

In the Sarcoma 180 program over 2,000 compounds have been screened. Of these eight have yielded the highest inhibition (+), and approximately forty have shown a weaker or intermediate inhibition (±). All others have been negative (−). The gradings are explained in the footnote to Table IV. This table contains representative results on a few of the types of compounds under study. The known antibiotics have shown little or no activity. A material from cultures of Aspergilli, however, has shown appreciable ability to inhibit Sarcoma 180.[25,64] The purines and pyrimidines presented in the table are inactive but a few others have shown activity out of many tested for possibility of interference with nucleoprotein metabolism. The best results have been obtained with the folic acid analogs and the nitrogen mustard type of compound. It is of interest that several compounds in the latter category are more effective in experimental animals than methyl bis(β-chloroethyl) amine used so extensively in the clinic. A-Methopterin, (4-amino-N^{10}-methyl pteroylglutamic acid), has appeared to be the best of the antifolics tested against Sarcoma 180. However, it is evident that it is inadequate in several respects. Figure 7 shows an area diagram of the effect of A-Methopterin in inhibiting the development of Sarcoma 180. The diagrams represent areas of individual tumors measured at one-week intervals. It illustrates the lack of permanent effect for the tumors resumed growth during the second week. Treatment was stopped at the end of the first week. In Table V are summarized the inhibitions of Sarcoma 180 obtained at different dose levels of A-Methopterin. The dose range between a toxic level and a minimum effective level indicates a small therapeutic index, a second defect. Other experiments[65] have revealed that the compound has a decreased effect when initiation of its administration is delayed

* Determination of the effect of the compound by measuring the tumors has been considered more reliable for Sarcoma 180 than use of survival time for in our experience this tumor has grown rather uniformly but the survival time has been quite irregular. It has varied from approximately twelve to sixty days in a survey of controls for one year.[63] Weighing of the tumors has been considered a time-consuming refinement unnecessary in a preliminary screening study. It is useful in providing a more accurate description of the results on selected effective compounds.

TABLE IV

STUDIES ON THE INHIBITION OF SARCOMA 180 IN THE MOUSE

Compound	Dose Level mg./kg./day	Toxicity*		Results†
		Relative Weight Change	No. of Deaths per Test Group	
Folic Acid and Analogs				
Folic acid	150	$\frac{-\frac{1}{2}}{+2}$	0	−
Aminopterin	0.2	$\frac{-1}{+2}$	0	+
A-Methopterin	1.5	$\frac{-1}{-1\frac{1}{2}}$	0	+
Amino-An-Fol	35	$\frac{-2\frac{1}{2}}{+2}$	1	± to +
Aminoteropterin	0.3	$\frac{-2}{+1}$	1	± to +
Teropterin	2000	$\frac{-1}{+3}$	0	−
Nitrogen Mustards and Related Compounds				
Methyl bis(β-chlorethyl)amine	0.8	$\frac{-3\frac{1}{2}}{-1\frac{1}{2}}$	1	±
Propane [1,3-bis(bis-β-chloroethyl)amine]	1.0	$\frac{-4}{+\frac{1}{2}}$	1	±
Dibenzyl-β-chloroethylamine	28	$\frac{+6}{+1}$	2	−
N,N-bis(β-chloroethyl)aniline	100	$\frac{-1\frac{1}{2}}{+2}$	0	±
2,4,6-Tris(ethyleneimine)-s-triazine	0.75	$\frac{-3}{-1}$	0	+
3-bis(β-chloroethyl)aminomethyl-4-methoxy-methyl-5-hydroxy-6-methyl pyridine dihydrochloride	7.5	$\frac{-3}{-1\frac{1}{2}}$	1	+
Carbamates				
Urethane	1000	$\frac{-1\frac{1}{2}}{+1\frac{1}{2}}$	1	±
Tetramethylene diurethane	250–300	$\frac{+4}{+2\frac{1}{2}}$	1	−
Ethyl-N-phenyl carbamate	50	$\frac{0}{+\frac{1}{2}}$	0	−
N-Phenyl isopropyl carbamate	32	$\frac{+\frac{1}{2}}{+\frac{1}{2}}$	0	−
Antibiotics				
Clavacin	2.5–5.0	$\frac{0}{+2}$	0	− to ±
Citrinin	60	$\frac{+3}{+3}$	0	±
Gliotoxin	2–3	$\frac{-\frac{1}{2}}{+\frac{1}{2}}$	1	− to ±

TABLE IV (*Continued*)

Compound	Dose Level mg./kg./day	Toxicity*		Result†
		Relative Weight Change	No. of Deaths per Test Group	
Antibiotics (*Continued*)				
Aspergillic acid	64	$\frac{+2\frac{1}{2}}{0}$	1	−
Actinomycin	0.05	$\frac{-4}{-\frac{1}{2}}$	2	±
Penicillin G	5000	$\frac{+2\frac{1}{2}}{+1\frac{1}{2}}$	0	−
Actidione	15	$\frac{-\frac{1}{2}}{-\frac{1}{2}}$	0	−
Chloromycetin	1000	$\frac{+3}{+\frac{1}{2}}$	0	−
Streptomycin	1000	$\frac{+3}{+3}$	1	−
Aureomycin	63	$\frac{+\frac{1}{2}}{+\frac{1}{2}}$	1	−
Polymyxin B	10	$\frac{-\frac{1}{2}}{+\frac{1}{2}}$	1	−
Purines and Pyrimidines				
2-Amino-4-methyl pyrimidine	750	0	0	−
Adenine	350	$\frac{+1}{+1\frac{1}{2}}$	2	−
2,4,6-Trichloropyrimidine	15	$\frac{-2}{0}$	0	−
2,4-Diaminopyrimidine	200	$\frac{-2}{-\frac{1}{2}}$	0	−
8-Azaguanine	150	$\frac{+3\frac{1}{2}}{+2}$	0	−
2-Amino-4-hydroxy-6,7-diphenyl pteridine	500	$\frac{+3}{+\frac{1}{2}}$	0	−
Miscellaneous				
4-Methyl-7-hydroxy coumarin	313	$\frac{-3}{+\frac{1}{2}}$	1	−
Coumarin	63	$\frac{-3}{+1\frac{1}{2}}$	0	−
1,4-Naphthoquinone	4	$\frac{+\frac{1}{2}}{+2}$	0	−
Malononitrile	15	$\frac{+1}{+3}$	0	±
Succinonitrile	5	$\frac{+3}{0}$	1	−
Diasone-(disodium formaldehyde sulfoxylate diamino diphenyl-sulfone)	500–600	$\frac{+3\frac{1}{2}}{+2}$	1	±
2-Aminofluorene	150	$\frac{+3\frac{1}{2}}{-\frac{1}{2}}$	1	−

TABLE IV (*Continued*)

Compound	Dose Level mg./kg./day	Toxicity*		Result†
		Relative Weight Change	No. of Deaths per Test Group	
Miscellaneous (*Continued*)				
2-Acetylaminofluorene	125	$\frac{+2\frac{1}{2}}{0}$	0	−
Methyl cholanthrene	500	$\frac{+3\frac{1}{2}}{0}$	2	− to ±
Methoxy pyridoxine	50	$\frac{0}{+\frac{1}{2}}$	2	−
Desoxy pyridine	128	$\frac{+4\frac{1}{2}}{+1}$	1	±
2-Aminoethyl sulfonic acid	250	$\frac{0}{+2}$	0	−
β-Naphthoxyacetic acid	32	$\frac{-1}{+2\frac{1}{2}}$	0	±
Nile blue	64	$\frac{+1}{+2}$	0	−
2,4-Diamino-6-(2-β-hydroxyethoxy-5-arsonoaniline)-s-triazine	125	$\frac{+\frac{1}{2}}{+1}$	0	−

* The fraction expressing the relative weight change contains the average weight change in gm./mouse of the test group in the numerator and similar data for the control group in the denominator. The number of deaths represent the number dead per test group of five. These are representative of a number of repeated tests. Some deaths may be due to injection accidents.

† The results of inhibition of Sarcoma 180 are expressed as follows:

−, No effect, when the largest diameter was ¾ or more of the diameter of the control tumors,

±, Slight inhibition, when the diameter was ¼ to ¾ of the diameter of the control tumors,

+, Marked inhibition, when the diameter was ¼ or less of that of the control tumors; actually, the volume of such a tumor would be $\frac{1}{64}$ or less of the control if tumors may be considered spherical.

TABLE V

INHIBITION OF GROWTH OF SARCOMA 180 BY DIFFERENT AMOUNTS OF A-METHOPTERIN

Dose mg./kg./day	No. of Mice	Deaths during Treatment Per cent	Relative Effect on Tumor			
			No. of Groups of 5 Mice Showing			
			Inconclusive Results*	Marked Inhibition	Slight Inhibition	No Effect
2.5	30	47	2	4†	0	0
2.0	75	19	2	9	4	0
1.5	155	7	0	16	11	4
1.0	40	5	0	1	4	3

* Results were considered inconclusive when three or more mice died in any group of five; other gradings as defined in Table IV footnote.

† Means four groups of five mice each showed marked inhibition at this level.

until there is little or no effect observed on Sarcoma 180 permitted to grow seven days prior to treatment.

In any chemotherapy screening program conditions of the study involve recognized risks even though the deficiencies may not

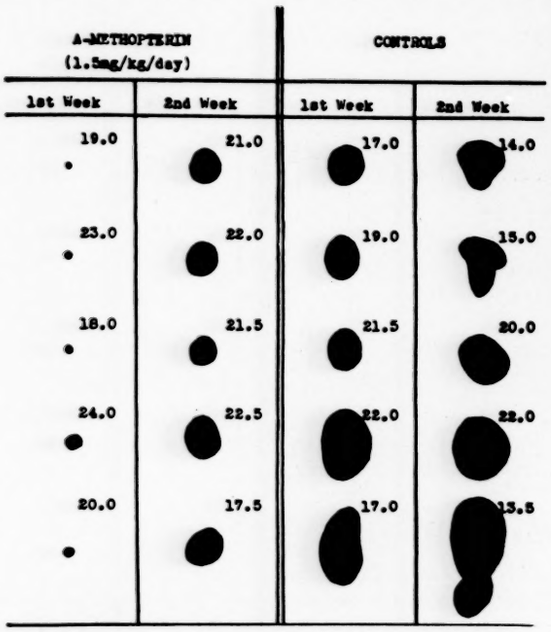


FIG. 7. Area diagrams of tumors (sarcoma 180) in mice treated with 4-amino-N-methyl pteroylglutamic acid (A-Methopterin) compared with untreated controls.

be calculable. Use of numerous tumors and various conditions represents one way of insuring the greatest usefulness of an experimental cancer chemotherapy program. We have employed a tumor spectrum including rat and mouse tumors of diverse cell types. Some grow rapidly and others slowly. In Table VI are presented the results[27,66] obtained with a few compounds in inhibition studies similar to those made in the Sarcoma 180 screening program. It is apparent that the compounds affect the tumors differently and that extreme differences in sensitivity to any selected compound may be found among the tumors; for example, rat sarcoma 39 is affected markedly by Aminopterin (4-amino pteroylglutamic acid) (Fig. 8) whereas under similar conditions the Flexner-Jobling carcinoma and at least one mouse tumor are not measurably inhibited. The spectrum study reveals that

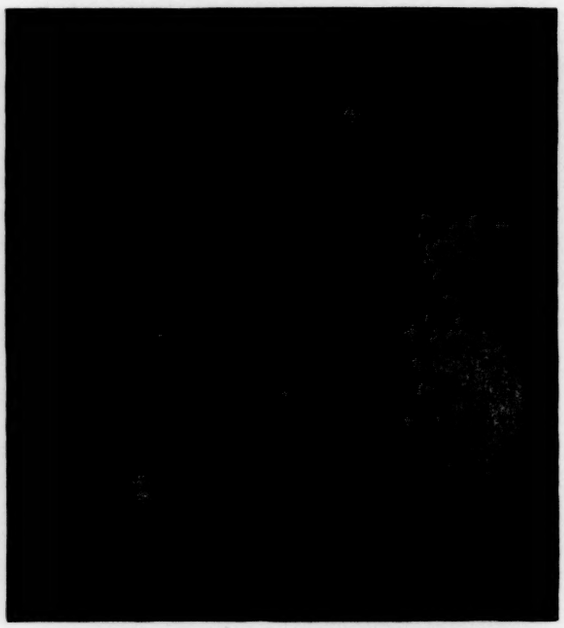


FIG. 8. Rats bearing sarcoma 39 implanted twenty-one days previously. The rat on the left is an untreated control; the rat on the right received 4-amino pteroylglutamic acid (aminopterin) 0.25 mg./kg./day for six days starting six days after implantation of tumor.

mammary adenocarcinoma EO771 is the only tumor listed in Table VI which is inhibited by 8-azaguanine.[67] The various examples in the table illustrate well the differences in susceptibility of various tumors to a number of compounds with known antitumor activity. It makes apparent the possibility of missing some compounds with antitumor activity because of the characteristics of the tumor chosen for a screening study and it also indicates the hazards in generalizing on antitumor activities possessed by any material.

In addition to the aid rendered by use of a spectrum of tumors, screening programs should be supplemented by specialized studies. Certain compounds or classes of compounds may present special requirements for their best evaluation. With current interest in the test of hormones it may be desirable to seek tumors of the endocrine system for screening materials which directly or indirectly are capable of affecting hormone balance. Some tumors which have been reported[68,69] may be more useful in that respect than the tumors presented

in Table VI. It is encouraging to note that the reported activity of cortisone against human lymphosarcoma[79] was foreshadowed by observations in a mouse lymphosarcoma[71] recently supported by similar results in two other lymphosarcomas.[72]

to be required on a larger scale with the development of newer chemotherapeutic agents, at least until outstandingly effective substances are discovered.

Attention has recently been redirected to the use of biologic entities to combat

TABLE VI

INHIBITING EFFECT OF COMPOUNDS IN A SPECTRUM OF MOUSE AND RAT TUMORS

Compound	Dose mg./kg./day	Mouse Tumors						Rat Tumors		
		Crocker Sarcoma 180	Lewis Sarcoma T241	Adeno-carcinoma EO 771	Harding-Passey Melanoma	Wagner Osteogenic Sarcoma	Patterson Lympho-sarcoma	Flexner-Jobling Carcinoma	Sarcoma 39	Walker Carcino-sarcoma 256
Methyl bis(β-chlor-ethyl)amine.HCl	0.5	−	−	−	−	± to +	−	++	+++	++
3-Bis(β-chloroethyl) aminomethyl-4-methoxy-methyl-5-hydroxy-6-methyl pyridine. 2HCl	5.0	±	−	±	−	++	±	+++	+++	+++
8-Azaguanine	75	−	−	++	−	−	−	−	−	−
2,6-Diaminopurine	60	−	−	−	−	−	−	−	±	
Triethylene melamine	0.25	±	−	−	−	+	− to ±	+++	+++	+++
4-Amino pteroyl-glutamic acid	0.25	++		+	±	−	++	−	+++	+++

Grading of Tumor Inhibition:
− Growth more than ¾ the average diameter of the controls.
± Growth from ½ to ¾ the average diameter of the controls.
\+ Growth from ¼ to ½ the average diameter of the controls.
++ No growth or growth to ¼ average diameter of the controls.
+++ Destruction of tumor.

It is conceivable that some materials would reveal activity only if administered more frequently than once or twice daily. One method of accomplishing this objective is incorporation of the test material in the diet. The material must be stable under these conditions; stability is, of course, a consideration under conditions of use when materials are injected. Manipulation of the diet is also a factor to be weighed when tests are made with substances which it is hoped will be active because of some antimetabolite activity. With materials as effective as some of the folic acid analogs[73] this has not been necessary but with weaker antagonists the best results have been achieved using a diet low or deficient in the metabolite.[15,74] Recent studies have also suggested the desirability of combining one effective material with another.[75] Studies on the concomitant use of two chemotherapeutic agents as well as the combination of chemotherapy and radiation[1] would appear

tumors. Viruses have been shown to be effective against tumors.[76,77] The strongest actions on the tumors in mice have been achieved with viruses pathogenic for the host as well as the tumor. The implications of the results have required initiation of a special program which is seeking to obtain a tumor-destructive strain of virus nonpathogenic to the host either by a mutation of the pathogenic virus or through a survey of other viruses which might possess the desired characteristics.

Mechanism of action studies are a natural consequence of exploitation of the fruits of experimental cancer chemotherapy studies. The unique activity of urethane in leukemia has made it an object for study with modern technics employing tracer experiments.[78] The finding of rapid breakdown of most of the injected urethane increases interest in further studies of its mode of action. While mechanism of action studies may follow the discovery of new

antitumor substances, it is well recognized that therapy may be based upon certain biochemical activities of a tissue. The use of radioactive iodine in the treatment of carcinoma of the thyroid illustrates this approach. Although this is an unusually specific physiologic process adapting itself well to a unique therapy, it suggests similar utilization of any newly developed knowledge of more or less specific biochemical properties of any tumor.

SUMMARY

Some approaches to experimental cancer chemotherapy have been presented. The value of a multiple approach is emphasized.

Evidence is accumulating to indicate the usefulness of animal studies in selecting compounds and other materials for study in human malignancy. Not only can animal trials discover new compounds for clinical evaluation but they may also provide a relatively rapid and economical method of determining the order of clinical testing of compounds to be selected from a large series of related compounds.

It is possible to say that, despite the many problems and slow progress in experimental cancer chemotherapy, the increasing availability of fundamental information and the practical achievements already accomplished, though still of limited scope, provide hope for ultimate success.

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Case Reports

Cirrhosis of the Liver Following Infectious Mononucleosis*

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WITHIN recent years there has been increasing awareness of involvement of the liver in the course of infectious mononucleosis. The clinical similarity between infectious mononucleosis and infectious hepatitis has doubtless been the main stimulus to studies of this subject. Our present knowledge rests upon four types of studies, namely, chemical, clinical, liver biopsy and postmortem.

Chemical aberrations suggestive of liver dysfunction have been studied by Cohn and Lidman,[1] DeMarsh and Alt,[2] Carter and MacLagen,[3] Shay, Berk and Siplet,[4] Peterson,[5] Gall,[6] Rapaport,[7] Ernst and Dotti,[8] Evans,[9] Iverson and Raaschou,[10] and Brown, Sims, White and Clifford.[11] The procedures used by these investigators include the thymol turbidity test, thymol flocculation test, bromsulfalein excretion, alkaline phosphatase determination, prothrombin time, serum protein determination (including a few electrophoretic pattern studies), cephalin-cholesterol flocculation test, serum bilirubin determination, icterus index values and colloidol gold reaction of serum. These reports plus personal observations, as yet unpublished,[12] in a series of twenty-four cases persuade us to accept as fact that the liver is involved in almost all, if not all, cases of infectious mononucleosis.

The second approach to this problem has been less direct, namely, demonstration of the frequent association of jaundice with infectious mononucleosis. Mackey and Wakefield[13] are generally credited with having reported the first such case in 1926 but priority belongs to Downey and McKinlay[14] who in 1923 described one case of jaundice among their nine instances of "acute lymphadenosis with lymphocytosis." Since then numerous similar case reports have appeared in the literature.[7,11,15–27] We are not completely convinced that all of these are authentic cases of infectious mononucleosis. We have not attempted to review completely the world literature in this regard. Boger[23] in reporting his case in 1944 listed it as the twenty-seventh reported to that time. Spring[25] in presenting his five cases in 1944 stated that, "Partial survey of the literature indicates that only about 35 cases of infectious mononucleosis with jaundice have been reported." Of considerable interest is the statement by Glanzman[28] in 1930 that icterus in infectious mononucleosis is due to a diffuse hepatitis and not to hilar node swelling. Although he mentioned that anatomic studies to support his contention were lacking, in a footnote he does cite a thesis of A. H. DuBois (Geneva, 1929) concerning an autopsied fatal case of a twenty-six year old male with monocytic angina in whose liver were found small groups of mononuclear infiltrations in the portal spaces and areas of necrosis with disappearance of hepatic cells. This patient died of empyema.

The third approach has been via biopsy study of the liver. Kilham and Steigman,[29] van Beek and Haex,[30] Bang and Wanscher,[31] and Davis, MacFee, Wright and Allyn[32] have reported on biopsies of the liver in a total of seven cases. In six cases the biopsies

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were by aspiration; in the last report the biopsy was obtained at operation for a ruptured spleen. The changes are those of an acute hepatitis, as in acute infectious hepatitis of a similar stage, i.e., patchy degenerative parenchymatous changes and lymphocytic and leukocytic infiltration in the portal areas. Van Beek and Haex performed a second biopsy on their patient after three and a half weeks when he was ambulatory. This revealed normal liver tissue. These studies are particularly important because they explode the long-held but untenable thesis that jaundice and liver damage in infectious mononucleosis are due to pressure and obstruction by lymph nodes around the porta hepatis and demonstrate clearly that the liver damage is primarily parenchymal.

This brings us to the fourth and possibly most significant series of studies on this subject, postmortem examinations. The first reference to a fatal case, possibly infectious mononucleosis, is by Korsakoff[33] in 1905. This patient, a five year old boy with glandular fever who died of nephritis, was autopsied but the liver is not specifically mentioned in the report. In 1927 Haken[34] described three cases of monocytic angina in children. At autopsy no liver lesions other than stasis and fatty change were mentioned. However, the diagnoses here are not clearly established. At least one of these patients had diphtheria. DuBois'[35] report from Geneva in 1930 (to which Glanzman[28] was referring) is the first which presents postmortem anatomic evidence of an acute hepatic lesion. In 1939 Thomsen and Vimtrup[36] presented autopsy data on two of six fatal cases from Denmark. In their first case the liver grossly appeared hyperemic with normal markings. The portal lymph nodes and the nodes along the common bile duct were swollen. Microscopically, the liver was described as normal except for many lymphocytes in the periportal tissue. In their other case the liver appeared normal grossly. Microscopically, there was abundant infiltration with mononuclear cells in the periportal spaces. In 1942 Jersild[37] presented postmortem data on another case from Denmark and briefly referred to the liver as not remarkable.

Since then in the United States and Canada there have been thirteen additional autopsy studies. Ziegler[38] in 1944 described the case of a twenty-two year old female with proven infectious mononucleosis who died in the fourth week from rupture of the spleen. Histologically, the liver revealed an acute hepatitis with extensive perilobular cellular infiltrations, as well as scattered foci, with destruction of most of the liver cells in these foci of infiltration. Liver cells with mitotic figures surrounded these cellular infiltrates. The sinuses were edematous, separating many of the Kupffer cells from adjacent liver cords. The endothelial cells of sinuses and blood vessels were swollen. The small bile ducts were lined by swollen epithelial cells so that their lumina were diminished.

Allen and Kellner[39] reported the case of a twenty-three year old Air Force pilot who died in a crash thirty-one days after the onset of proven infectious mononucleosis and two weeks after the remission of symptoms. The histology of the liver was similar, in general, to Ziegler's description.

Custer and Smith[40] presented the data on nine autopsy studies (the case of Allen and Kellner is included in this paper from the Army Institute of Pathology) and reported that the liver lesion was essentially a periportal hepatitis hardly distinguishable from liver changes in the milder cases of epidemic hepatitis as observed at biopsy. Necrosis of liver cells was observed in only one of the fatal cases. Seven of these nine cases had been described in previous reports.[39,41,42]

Fisher[43] described the cases of two Canadian soldiers who died of spontaneous splenic rupture. At autopsy the liver lesions were similar to those revealed by biopsy studies as described before.

Most recent has been the report by Dolgopol and Husson[43a] of a nineteen year old female office worker who died of neurologic complications after a nine-day

illness. The histologic examination of the liver showed heavy mononuclear cell infiltrations in the portal areas and to a lesser extent in the sinusoids.

Thus, the accumulated evidence fairly conclusively indicts the liver as playing an active role in the disease we call infectious mononucleosis. Isaacs[44] talks of chronic infectious mononucleosis but does not support his thesis with liver function studies. Others[1,7,45] allude to a chronic hepatitis complicating infectious mononucleosis. However, no demonstration of the possible logical conclusion in this sequence of events, cirrhosis of the liver, has yet been forthcoming. The case we shall now report in detail is, we believe, the first documented instance of this complication.

CASE REPORT

First Beth Israel Hospital Admission October 31, 1945 to November 15, 1945. T. K., a twenty-four year old white male employed as a cafeteria counterman, was admitted to Beth Israel Hospital on October 31, 1945, because of jaundice and epigastric pain. He stated that about five weeks previously he vomited after meals. The vomiting continued for two and a half weeks. He then suffered two bouts of diarrhea, each lasting one to two days. Jaundice set in three weeks before admission and persisted, increasing until admission. For the previous four days he felt pain in the epigastrium radiating toward the right, not relieved by any measures nor aggravated by any particular foods. At the time of admission he felt only soreness in the right upper abdomen. During these five weeks he had about ten spontaneous nosebleeds. His weight decreased from 140 to 108 pounds. There was no itching. For three weeks his stools were light yellow and his urine dark red. He stated that his appetite had remained fairly good.

He had no living relatives. His father had died accidentally and his mother had died of kidney disease. The patient had shifted for himself since he was seventeen. He denied drinking any liquor.

On admission his temperature was 101°F., pulse rate 72 beats per minute, respirations 22 per minute and blood pressure 120/65. He was a well developed, thin, intensely jaundiced young adult male, complaining of epigastric soreness. There were tattoo marks on both arms. The skin and scleras were deeply jaundiced. An abscess was noted behind the left ear. In the left naris there was evidence of recent bleeding plus a yellowish exudate. The posterior pharynx was injected. Many small discrete lymph nodes were felt in both anterior and posterior cervical chains. Axillary, epitrochlear and inguinal nodes were palpable. The heart and lungs were normal. The epigastrium was tender. The liver was tender and enlarged to four fingerbreadths below the costal margin. The spleen was enlarged to two fingerbreadths below the costal margin. No costovertebral tenderness was elicited.

Pertinent data of urine analyses, blood counts, erythrocyte sedimentation rates, blood chemical examinations and prothrombin times are listed in Table I. No abnormal lymphocytes were noted in the smear of the peripheral blood. Bile and urobilinogen were present in the feces. No occult blood was found. Blood Wassermann, Kahn and Kline examinations were negative. The serum heterophile agglutination with sheep cells was positive 1:100 on the third hospital day. (Table II.)

The diagnostic impressions on admission were infectious hepatitis or infectious mononucleosis. The latter diagnosis was supported by a rise in the heterophile titer to 1:200 on the sixth hospital day. On the thirteenth hospital day the titer rose to 1:800, substantiating this diagnosis. No absorption tests were performed. The temperature remained normal after the first day. The patient complained frequently of epigastric pain. He was constipated while in the hospital. His diet was high in protein and carbohydrate, low in fat. He was given 1 gm. of choline chloride by mouth three times daily and 10 mg. of vitamin K by vein daily. He was allowed out of bed after the second day. A sternal marrow puncture on the third day revealed a hypercellular marrow of normal composition. The posterior cervical and axillary lymph nodes were increased in size and number on the sixth day. By the fourteenth day the liver was three fingerbreadths and the spleen one and a half fingerbreadths below the costal margin. He still complained of right abdominal pain. Jaundice was receding. On the sixteenth day he requested discharge.

At this point in the case report we insert information obtained from another hospital several years later when it was learned that he had been admitted there six weeks before this

TABLE I

LABORATORY DATA IN CASE REPORTED

Date	Urine	Blood Count									Erythrocyte Sedimentation Rate Mm./Hr.	Chemical Examination of Blood										
		R.B.C. Millions/cc.	Hemoglobin		W.B.C. per cc.	Differential						Proteins gm. %		Bilirubin Mg. %	Icterus Index Units	Thymol Turbidity Units	Cephalin flocculation at 48 Hr.	Alkaline Phosphatase Units	Cholesterol in Mg. %		Bromsulfalein % Ret'd. in 45 Min.	Prothrombin Time	
			%	Gm.		Poly.	Staff	Lymph.	Eos.	Mono.		Albumin	Globulin						Total	Esters		Sec.	% Normal
First Hospital Admission 10/31/45–11/15/45																							
11/1/45	1.011 ALB.-0 bile + Urobilinogen +	3.50	71	11	5900	57	5	29	2	4	15	3.2	3.1	25	114	....	4+	...	185	65	...	19.9	....
11/6/45	0 for leptospira	4.20	71	11	5500	65	0	25	2	5	..	...	...	....	81	....	.....	...	169	63	...	....	....
11/8/45	.....	....	..	..	.....	..	..	..	..	..	18	...	...	....	63	....	3+	...	...	..	...	....	....
11/13/45	Bile + Urobilinogen +	3.85	71	11	7600	64	3	26	4	3	1	3.8	3.4	....	38	...	.....	...	...	..	...	....	....
Second Hospital Admission 11/19/45–12/4/45																							
11/20/45	1.030 Bile + urobilinogen +	3.80	71	11	5400	61	9	23	3	4	..	4.1	3.0	....	27	....	....	4.8	188	94	...	....	....
11/27/45	Bile 0 Urobilinogen 0	3.45	71	11	4700	52	2	31	5	10	10	...	...	....	29	....	2–3+	5.8	...	..	...	19.6	....
12/3/45	Bile 0 Urobilinogen 0	....	..	..	.....	..	..	..	..	..	..	...	...	....	36	....	2+	...	...	..	...	....	....
Third Hospital Admission 8/19/48–9/10/48																							
8/20/48	1.026 Bile 0 Urobilinogen 1:20	3.93	61	9.5	7750	41	0	52	2	4	..	4.3	3.2	0.78	...	21	Trace	6.2	127	62	...	....	....
8/23/48	.....	....	..	...	.....	..	..	..	..	..	35	...	...	....	...	....	3–4+	...	...	..	10	15.2	>30
8/31/48	1.030	3.53	61	9.5	.....	..	..	..	..	..	..	...	...	0.26	...	20.7	.....	5.1	...	..	...	....	....
9/2/48	.....	....	..	...	.....	..	..	..	..	..	30	...	...	0.12	...	23.6	3+	1.9	...	..	...	....	....
Fourth Hospital Admission 11/26/48–12/23/48																							
11/27/48	1.022	3.45	55	8.5	10650	66	28	3	1	2	19	4.6	2.7	1.6	...	13.5	3–4+	...	139	..	...	19.3	16.4
12/2/48	Galactose tolerance test normal (3.1 gm. in 5 hours' urine)	....	..	...	4700	58	2	31	3	6	..	...	...	0.29	...	....	.....	...	138	24	14	16.7	24.1
12/9/48	.....	....	..	...	.....	..	..	..	..	..	..	4.6	3.4	....	...	20	.....	...	...	..	7.5	14.6	>30
12/20/48	Bile 0 Urobilinogen 1:160	....	..	...	.....	..	..	..	..	..	..	...	...	....	...	....	.....	...	...	..	...	16.1	26.5

first admission to Beth Israel Hospital. We learned that on September 15, 1945, he had entered this other hospital with a history of generalized weakness, loss of appetite and diarrhea of two days' duration. He had noticed that his skin and eyes had become yellow and his urine dark red. He had never had a similar illness. His temperature was 100°F. The abdomen presented a nodular mass in the left upper quadrant which was hard, non-painful and appeared to be in the abdominal wall. Liver and spleen were not felt. Gallbladder roentgenograms showed no gallstones and no shadow of a gallbladder. By roentgenogram the spleen was slightly enlarged, the edge of its shadow being seen just below the last rib.

By his third hospital day the liver was two fingers below the costal margin. He was studied from the standpoint of acute catarrhal jaundice. The urine on admission had a trace of albumin, occasional hyaline casts, granular casts, two to three white cells; the bile was strongly positive and urobilinogen positive in 1:40 dilution. Repeated urine examination showed urobilinogen positive in 1:320 dilution. Gradually, the urine became negative and by the time of discharge on October 26, 1945, it contained only four to five white cells. On admission the red blood count was 4,050,000 per cu. mm., hemoglobin 86 per cent. The white blood cell differential count was 69 per cent polymorphonuclears, 30 per cent small mononuclears and 1 per cent large mononuclears. The icterus index on admission was 100 units, with repeated readings of 67 and 40. Sedimentation rate on admission was 24 mm. per hour, with repeated readings of 24, 54, 10 and 15. Serum albumin was 4.6 gm. per cent, serum globulin 1.5 gm. per cent. Bromsulfalein test showed 40 per cent of dye retained at thirty minutes. Stools were bile-positive.

On October 26, 1945, he decided to go home and left the hospital on release. The jaundice had not cleared up. The liver remained enlarged to the level of the umbilicus and felt hard and irregular. It was palpable all the way across the abdomen up under the ensiform cartilage on the left side. The spleen was palpable and firm but not hard. Diagnosis on discharge was "catarrhal jaundice." There were some doubts because this appeared to be a long illness for a simple "catarrhal jaundice" and because a history of malnutrition had been obtained.

Second Beth Israel Hospital Admission November 19, 1945 to December 4, 1945. In the four days that elapsed since leaving the hospital he felt well until the day of readmission when severe pain in the right side of the abdomen and tenderness there on palpation developed. When these pains progressed he returned to the hospital.

On admission temperature was 100.4°F., pulse rate 100 beats per minute and respirations 28 per minute. Findings were essentially the same as at the time of discharge four days previously except that the jaundice was diminished. The liver was firm, tender and palpable three finger-breadths below the costal margin. The spleen was barely palpable.

TABLE II
RESULTS OF SHEEP CELL AGGLUTINATIONS IN CASE REPORTED*

Hospital Admission	Date of Test	No. Days after Onset of Initial Illness	Titer
First	11/2/45	50	1:100
	11/5/45	53	1:200
	11/12/45	60	1:800
Second	11/23/45	71	1:50
Third	8/20/48	..	1:50
	8/24/48	..	1:50
Fourth	12/2/48	..	1:<25

* The technic used was the centrifuge method. The tubes, when finally prepared, were allowed to stand at room temperature for ten minutes, then centrifuged at 500 R.P.M. for two minutes and then read by flipping the bottom of each tube with the index finger. Final total volume in each tube was 1.0 cc., consisting of 0.5 cc. of the serum dilution in saline (1:25, 1:50, 1:100, etc.) plus 0.5 cc. of the 2 per cent sheep cell suspension. The titer as expressed is the serum dilution *prior* to the final addition of the sheep cells. If the final titer were recalculated with the sheep cell volume included, listed titers should be doubled.

Pertinent laboratory data are listed in Tables I and II. On the fifth hospital day the serum heterophile agglutination titer was 1:50. With dye administered orally gallbladder roentgenograms showed faint filling but no stones were seen. Gastrointestinal roentgenographic series was negative.

After the first day the temperature remained normal. The patient experienced severe epigastric pains unrelieved by nitroglycerine, atropine, gastric lavage, malcogel, other antacids or sterile hypodermics. Relief was obtained only by demerol or other narcotics. He often complained of abdominal pain after smoking or walking about the ward. He signed himself out against advice sixteen days after admission.

It was later learned that in April, 1946, during

a three months' stay at a state institution he was admitted to the hospital for two days following a complaint of epigastric distress. Blood count, urinalysis and gastrointestinal roentgenograms were reported as negative. These were his only physical complaints during this period.

Third Beth Israel Hospital Admission August 19, 1948 to September 10, 1948. For several weeks he had noted malaise and weakness. His urine became dark one week previous to admission. During the three days before admission he had lost his appetite and complained of nausea, right upper abdominal and epigastric pains and vomiting. He had a chill two days before admission. On the evening of admission the abdominal pains had been very severe and he had fainted. Since his last admission he had suffered vague abdominal pains but no nausea, vomiting or jaundice. Recently he had lost some weight. He admitted that from the ages twenty to twenty-two (six to four years before) he had consumed a pint of whiskey daily but was drinking only beer previous to the current admission.

On admission his temperature was 99.2°F., pulse rate 92 beats per minute, respiratory rate 20 per minute, blood pressure 105/65. He was undernourished and appeared acutely ill. There was a suggestion of scleral icterus. The left nostril contained clotted blood. The pharynx was injected. The axillary, epitrochlear and inguinal lymph nodes were enlarged. The right upper abdomen and epigastrium were tender. A sharp, tender liver edge was felt three fingerbreadths below the costal margin. The tip of the spleen was felt on deep inspiration. It was firm, not tender. Neurologic status was normal.

Roentgenogram of the chest was negative. Roentgenogram of the abdomen revealed no calculi in the region of the pancreas. With double dose of priodax the gallbladder appeared normal by roentgenogram. Gastrointestinal roentgenograms were negative; no esophageal varices were seen. On the sixth hospital day when the heterophile agglutination titer of the serum was 1:50, the agglutinins were absorbed completely by guinea pig kidney and incompletely by boiled beef erythrocytes. Other pertinent laboratory data are listed in Table I.

He was treated with bed rest, high caloric diet with high carbohydrate and protein and moderate fat values, crude liver intramuscularly three times weekly and vitamin supplements. His temperature remained normal throughout his twenty-three days in the hospital except for one rise to 100°F. on the twenty-first day. He frequently suffered pains in the right upper abdomen and epigastrium. He manifested only slight improvement by the time he was discharged. The diagnosis was chronic hepatitis on the basis of infectious mononucleosis and possibly cirrhosis of the liver.

Fourth Beth Israel Hospital Admission November 26, 1948 to December 23, 1948. His complaints were loss of appetite, nausea, pain in the back and vomiting of two days' duration. For one week he had had a cough and running nose. The day before admission he vomited suddenly after lunch and three or four times thereafter. Sharp pain developed in the right loin recurring at twenty-minute intervals for several hours. The pain then became dull and aching in character and radiated across the back towards the left side. On the day of admission he vomited again and the pain continued. He had been eating well since the last hospitalization, drinking only beer and smoking a package of cigarettes daily. He had been employed as a busboy and counterman.

On admission his temperature was 102°F., pulse rate 106 beats per minute, respiratory rate 24 per minute, blood pressure 110/70. He was not jaundiced. There was generalized enlargement of the cervical, axillary, epitrochlear, inguinal and femoral lymph nodes but no tenderness. A mucopurulent nasal discharge was noted. The pharynx was reddened. His teeth were in a poor state of repair. At the left lower lung field, posteriorly, there were râles, dullness and diminished breath sounds. The liver was enlarged to three fingerbreadths below the costal margin in the mid-clavicular line. Its edge was firm and sharp. It was tender to palpation and percussion. The spleen was felt one fingerbreadth below the costal margin. Several spider angiomas were seen on the skin over the right side of the neck and over the manubrium sterni. Pectoral and axillary hair was absent.

Pertinent data regarding tests of the urine, blood counts and blood chemical values are listed in Table I. Serum heterophile agglutination titer was 1:less than 25. (Table II.) No varices were seen in roentgenograms of the esophagus.

He was again placed on a diet high in carbohydrate and protein plus vitamin supplements (including 10 mg. daily of vitamin K) and intramuscular liver injections. Methionine was given in 1 gm. dosage six times daily. He was kept in bed. His temperature returned to normal on the

second day. On the fifth day a tooth was extracted because of a periapical abscess which had been revealed by x-ray examination. On the eleventh hospital day an aspiration biopsy of the liver was performed. The histologic examination of this tissue showed disorganization of architecture with pseudolobule formation. There appeared to be an increase in rather young fibrous tissue. Periportal lymphocytic infiltration was dense. The diagnosis was cirrhosis of the liver. (Fig. 1.)

During the third week in the hospital he complained frequently of nausea and pains in the epigastrium and right upper abdomen. This was accompanied by occasional transient rises in temperature up to 100.4°F. and once up to 102°F. At the end of four weeks he signed out of the hospital against advice. The diagnosis at this time was cirrhosis of the liver.

FIG. 1. Liver needle biopsy showing pseudolobulation, with increase in fibrous tissue; azo-carmine stain, ×80.

COMMENTS

Diagnosis of Infectious Mononucleosis. It is entirely appropriate to ask whether the basic diagnosis of infectious mononucleosis was established in this patient. In the present state of our knowledge of this presumed virus disease—the causative agent is as yet unidentified—the diagnosis of infectious mononucleosis in any one case rests on three supports, namely, clinical, hematologic and serologic. There is no single typical clinical picture, as is well emphasized by numerous students of this disease.[46,47] In his original description of glandular fever in 1889[48] which was concerned with children as were most of the early reports[49] Pfeiffer described both the glandular and the so-called abdominal types. However, it is now well known that infectious mononucleosis is a disease of protean manifestations, anginose, glandular, pneumonic, abdominal, septic and cerebral. The clinical data in our patient certainly fit the disease. Complicating hepatitis is a common concurrent feature of the acute illness, as we have indicated in our opening paragraphs. In fact, on the basis of unpublished studies,[12] we are inclined to question the validity of a diagnosis of infectious mononucleosis unless accompanying hepatic dysfunction is proven.

Hematologically, the important usual findings are the appearance of atypical lymphocytes in the peripheral blood and an increase in the total number of lymphocytes. No atypical lymphocytes were observed in this patient. This may have been due to the fact that we first observed him more than a month after the start of his illness. A lymphocytosis of 52 per cent in the third period of hospitalization was the only observed increase in lymphocytes. This is a point of weakness in the diagnosis but not an excluding one. It is worth keeping in mind that in the only other seriously considered diagnosis, infectious hepatitis, these same hematologic findings are common.[50–54]

Our diagnosis derives its main support from serologic tests. A rising heterophile antibody titer, climaxed by a 1:800 titer on the sixtieth day of illness and subsequent fall to within normal limits, appears to be incontravertible evidence. (Table II.) Two important points may be made which emphasize this statement.

First of all, this titer has never been reached in infectious hepatitis, the only diagnosis other than infectious mononucleosis which merits serious consideration in this case. Eaton, Murphy and Hanford[55] reported a maximum titer of 1:160 in 9 per cent of 222 serum specimens from cases of virus hepatitis. When their titers are recalculated on the basis of final serum dilution, including the sheep cell suspension, this 1:160 titer becomes 1:320. Havens, Gambescia and Knowlton[56] examined serum from 508 patients with virus hepatitis (including thirty experimentally induced) and found a maximum heterophile antibody titer of 1:56 in sixteen of the cases (3 per cent incidence). Berk, Shay, Ritter and Siplet[57] performed heterophile studies in twenty-five cases of virus hepatitis. Their maximum titer was 1:56 and was found in three of the twenty-five cases (12 per cent incidence). Unpublished studies[12] on virus hepatitis have yielded an elevation of titer (i.e., above 1:56) in nine of thirty-six cases to date, with 1:448 in one case, 1:224 in four cases and 1:112 in four cases. To approach this problem from a slightly different aspect it is interesting to note that the heterophile antibody studies by Schultz[58] in diseases other than infectious mononucleosis (and not including infectious hepatitis cases) revealed an occasional elevation in titer up to 1:448 (in one case of tuberculosis, one case of sarcoma and one case of monocytic leukemia). Higher titers than this were reached only in her cases of infectious mononucleosis. A possible explanation for the relatively high antibody titers Schultz found in cases other than infectious mononucleosis lies in the method she used (readings of the tubes after two hours at room temperature and refrigeration overnight). As a rule this technic increases the titer by at least one dilution.[59,60]

The second important point about the titers in our case is the lengthy interval after the onset of illness when a significant titer appeared. It is generally stated that in infectious mononucleosis the heterophile antibodies usually appear early, i.e., within the first two or three weeks of illness.[61,62] However, this is not an absolute rule. In personal studies[12] on twenty-four cases significant elevations first occurred on the thirty-third day in one case and on the thirty-first day in another case. The average for all twenty-four cases was thirteen days after the onset of illness. Abrams[27] reported a case of infectious mononucleosis with intense jaundice of unusual duration (at least eleven weeks) in which the antibody titer was negative in the fifth week of illness, 1:28 at seven and a half weeks, 1:112 at eight and a half weeks and finally rose to 1:896 in the tenth week of illness. Abrams' case duplicates our own in many aspects. It is interesting that, in underscoring the extreme importance of this late-rising titer in establishing the diagnosis of infectious mononucleosis in his case, he admits that were the test not repeated over several months' period the diagnosis would undoubtedly have been infectious hepatitis. The same would have been true of our case.

Absorption tests with guinea pig kidney and boiled beef erythrocytes were not applied in this case at the time the significant titers were found. The absorptions performed three years after the initial illness, when the unabsorbed titer was low (1:50), yielded a reaction characteristic of normal sera and the reverse of that typically seen at the height of infectious mononucleosis. Since the so-called infectious mononucleosis antibodies rarely persist in the serum beyond six months after their initial appearance,[12,60–62] this finding of normal antibodies three years later is to be expected. However, all students of the subject agree that a titer of 1:800 hardly requires corroboration with absorption tests although of course it is always a desirable confirmation.

We believe, therefore, that the underlying diagnosis of infectious mononucleosis in this case is well founded.

Cirrhosis of Liver, Diagnosis and Probable Etiology. Our patient received careful clinical and laboratory study during the course of four periods of hospitalization extending over three years. The development of a

chronic hepatic lesion was clearly traced, probably from its inception. However, without microscopic examination no prediction regarding the actual histologic picture of the liver would be possible. Volwiler, Jones and Mallory[63] have recently underscored this important point.

The biopsy picture permits us to state with assurance that the chronic lesion here has progressed to cirrhosis. (Fig. 1.) The term cirrhosis is used to indicate an increase in fibrous tissue and distortion of the structure of the liver lobules. It is very tempting to enter into a discussion of diffuse hepatic fibrosis and of postnecrotic scarring and to attempt to place our case into one or the other category. This, however, would be presumptuous because our entire histologic study is of approximately 8 cu. mm. of liver tissue and we believe this is insufficient tissue upon which to make such nice classification. If we draw an analogy from late liver lesions in infectious hepatitis,[64] it might be reasonable to anticipate that the liver lesion following infectious mononucleosis should be of the massive necrosis type with ensuing postnecrotic scarring. However, in the material available for study in our case the fibrosis appears to involve each of the lobules to a similar extent, i.e., it resembles more the picture of diffuse hepatic fibrosis which commonly follows zonal necrosis or diffuse severe fatty change.

Even granting that some acute liver lesion may occur in infectious mononucleosis, should one overlook the possible etiologic role of the nutritional deficiency which probably antedated and likely followed the acute illness? In our concept of the pathogenesis of cirrhosis in this case both the acute infection and the deficient diet play important roles. Himsworth and Glynn[65,66] have introduced the terms "toxipathic" and "trophopathic" to connote two types of noxious stimuli to which we refer. A toxipathic lesion results from the direct action of a noxious agent, be it chemical or a living organism. A trophopathic lesion is due to deprivation of a factor essential to cellular life, be that oxygen or a nutriment. The virus (?) of infectious mononucleosis was the toxipathic stimulus; the deficient nutrition was trophopathic. Either noxious stimulus alone might have caused some of the hepatic damage. We believe that both, acting in concert, accelerated the appearance of this cirrhotic lesion. Possibly the liver was more vulnerable because of the effects of poor nutrition prior to the occurrence of the acute infection. Certainly the poor state of nutrition was a contributing factor in the rapid and downward course following the initial acute illness and may explain why no demonstrable improvement in liver function resulted despite reasonably good care during his hospital stays. (In the light of our present knowledge of liver involvement in infectious mononucleosis failure to enforce more complete bed rest during the first period of hospitalization was an exception to otherwise good care.) It appears likely that the progression over the course of many months was due to the original tissue damage (toxipathic stimulus) in an already impaired vital organ and that regeneration and repair were hindered by the deficient food intake (trophopathic stimulus).

It might be argued that the picture found at biopsy might have developed even if an attack of infectious mononucleosis had not supervened. To us, such a hypothetical supposition does not appear justifiable because acute liver damage due to virus disease unquestionably did occur and therefore cannot be dismissed as having no important bearing on the evolution of the hepatic lesion. In each case all insults to the liver must be accorded their proper etiologic weight.

It is apparent that the occurrence of permanent liver damage following infectious mononucleosis must be exceedingly rare, less common even than in infectious hepatitis. In both diseases the existence of primary liver damage is now proven. The primary liver lesions are similar. Posthepatitic cirrhosis has been repeatedly described.[64,67–75] The determining and causal factors have not been clearly established. By

analogy, cirrhosis might be expected, even if only rarely, after infectious mononucleosis. The poor diet of our patient might have served as the conditioning factor which made for progressive liver disease rather than restitution to normal, the usual sequence of the liver damage occurring in infectious mononucleosis.

SUMMARY

1. A case is presented of a twenty-four year old male with infectious mononucleosis in whom cirrhosis of the liver developed, proven by biopsy, while under observation over a period of three years during which there were four hospital admissions.

2. The relative etiologic roles of the acute infection and poor diet are discussed.

3. The chemical, clinical, liver biopsy and postmortem studies which form the basis for our present knowledge of liver involvement in infectious mononucleosis are reviewed.

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Nephrotic Syndrome with Exaggerated Premenstrual Water and Salt Retention*

RICHARD W. LIPPMAN, M.D.
Los Angeles, California

THE nephrotic syndrome, characterized by a degenerative renal lesion, proteinuria and edema formation, is not uncommon in the clinical practise of medicine. Neither is it uncommon to see water and salt retention during the premenstrual period in women, often resulting in a cyclical gain and loss of several pounds in weight.[1,2] The co-existence of these clinical situations, with water and salt retention at times causing a gain of 20 pounds in weight, however, is not common.

CASE REPORT

Mrs. M. G., a thirty-two year old woman, was last known to have had normal urine in 1939. On June 11, 1944, she had an itching rash with blebs on her right arm and face, said to have been poison oak dermatitis, which was treated with calamine lotion. She had never before had such a dermatitis. On June 25, 1944, she first noticed swelling of the ankles and, upon consulting a physician, was considered to be in cardiac failure for which she received treatment. There is no record of urine examinations at this time. She was in a sanitorium for four and one-half months and received digitalis, salyrgan, penicillin and a diet low in salt but high in protein. With this regimen she failed to improve and, in fact, the edema increased. From a normal weight of 148 pounds, she increased in weight to 189 pounds.

When first seen in the Clinic for Renal Diseases, Stanford University Medical School, on March 20, 1945, she weighed 182 pounds. There was marked, generalized, soft, pitting edema involving the legs and extending up the abdominal wall. There was no hydrothorax or ascites. The blood pressure was 125/95. Physical examination was otherwise entirely normal and no evidence of cardiac disease was found.

Laboratory examinations at this time, March, 1945, revealed a Wintrobe hematocrit value of 36, total serum protein concentration 3.4 per cent, serum urea concentration 37.1 mg. per 100 cc., serum creatinine concentration of 1.95 mg. per 100 cc. Urinary excretions for a twenty-four-hour period were: protein 13.8 gm., erythrocytes 1.5 million, leukocytes and epithelial cells 38 million, casts (hyaline and hyaline with fat) 2.4 million. These findings are substantially the same as those repeated many times during close observation in the Clinic. The diagnosis was not perfectly clear because of the precedent allergic dermatitis and because, as Addis has commented,[3] cardiac failure is often associated with the initial stage of glomerular nephritis. However, the consensus favored glomerular nephritis, degenerative stage.

Since March, 1945, she has been on a therapeutic regimen which prescribed a diet containing 50 gm. of protein and less than 2 gm. of sodium chloride per day, with a polyvitamin supplement. Her weight rose periodically and she received intravenous infusions of human pooled plasma or, later, of concentrated salt-poor human albumin on numerous occasions when the edema became uncomfortable and disabling, with the association of right hydrothorax. Usually she had a very prompt diuresis with relief of her discomfort. Sometimes the needle was hardly in the vein when the diuresis began although sometimes there was a lag of one or two days before the diuresis started.

In August, 1947, it was noted that she had been coming in for brief hospital admissions and plasma or albumin administration almost monthly, with uniformly good responses. But for the first time it was noticed that her menstrual period began while she was in the hospital.

* From the Department of Medicine, Stanford University School of Medicine, San Francisco, Calif., and the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, Calif.

Upon questioning the patient it was readily elicited that for the past year all the menstrual periods she could remember had occurred in the hospital at the time she was receiving plasma or albumin. The possibility of a relationship immediately came to mind, and it was decided to withhold further diuretic therapy while the patient carefully recorded her daily morning weight and charted her menstrual periods. Sodium chloride excretion could not be studied because the patient lived in a town at some distance. She has been followed for more than a year, with results of the first eight months being recorded in Figure 1.

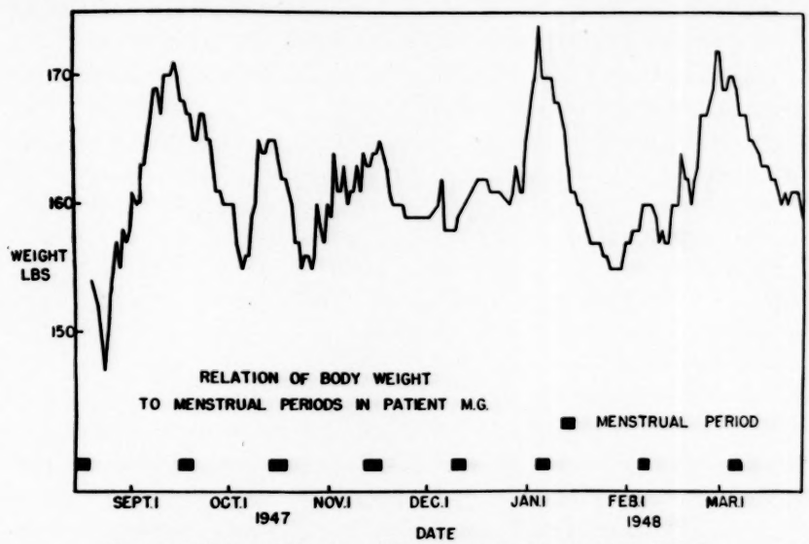

FIG. 1. Relation of body weight to menstrual periods in M. G.

In November, 1947, the patient's urine contained the following elements during a twenty-four-hour period: protein 12.6 gm., erythrocytes 2 million, leukocytes and epithelial cells 40 million, casts 0. The sediment contained many oval fat bodies. At this time the Wintrobe hematocrit value was 39, serum creatinine concentration 1.00 mg. per 100 cc. and blood pressure 130/90.

COMMENT

This case was included in a recent paper by Rytand[4] who discussed the association of poison oak dermatitis with the nephrotic syndrome. However, this case has additional interest because of the very wide weight fluctuations associated with premenstrual water and salt retention. For many months failure to recognize this circumstance resulted in repeated administration of plasma and albumin, with the belief that a very satisfactory therapeutic result was being obtained.

After the observation was made in this instance an effort was made to accumulate similar data in other patients with the nephrotic syndrome. It was extremely difficult to obtain the faithful persistence in recording data which was given us by M. G. over a protracted period. But in several cases followed for more than two months and two menstrual cycles no such striking weight changes occurred.

CONCLUSIONS

1. The premenstrual retention of salt and water may result in pronounced cyclical weight variations during the nephrotic state.

2. Such quasi-physiologic variations may confuse the interpretation of therapeutic results.

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Book Reviews

Diagnosis of Viral and Rickettsial Infections. Frank Horsfall, M.D., Editor. 153 pages. New York, 1949. Columbia University Press. *Price* $3.75.

This small volume presents to the clinician a concise and lucid survey of recent advances made by various investigators in viral and rickettsial infections. The thirty-two diseases included are discussed by well known authorities who have carried on original research in their respective fields. Available data concerning the pathogenic agent of each infection, its effect on the host and the immune responses of the host are summarized. The symposium includes laboratory descriptions of infectious mononucleosis, infectious hepatitis and atypical pneumonia, in addition to diseases of known etiology.

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Hematology. 1st ed., 915 pages. Cyrus C. Sturgis, M.D., Springfield, Ill., 1948. Charles C. Thomas. *Price* $12.50.

In this new textbook hematology is presented entirely from the point of view of pathologic states, beginning with anemias and including hemorrhagic diseases, white cell dyscrasias and allied lymphomatous states. A chapter is devoted to marrow puncture, a procedure which is now being used more widely. Laboratory procedures are not considered in detail, perhaps wisely, since they can be found elsewhere.

The amount of space given to historical aspects is unusual in modern monographs; the reason for this given by Dr. Sturgis in his preface is that "a scholarly knowledge of any branch of medicine is based upon a clear understanding of the principal advances and their sequential development . . . . " The historical accounts are long, but are valuable and delightful to the reader.

In stressing clinical aspects of hematology the author has drawn heavily on his long experience in the University of Michigan Hospital. Treatment is presented thoroughly and clearly. The use of large type in the text, and especially in outlines and classifications, makes reading easier. Placement of the references at the foot of the page is a convenience to the reader.

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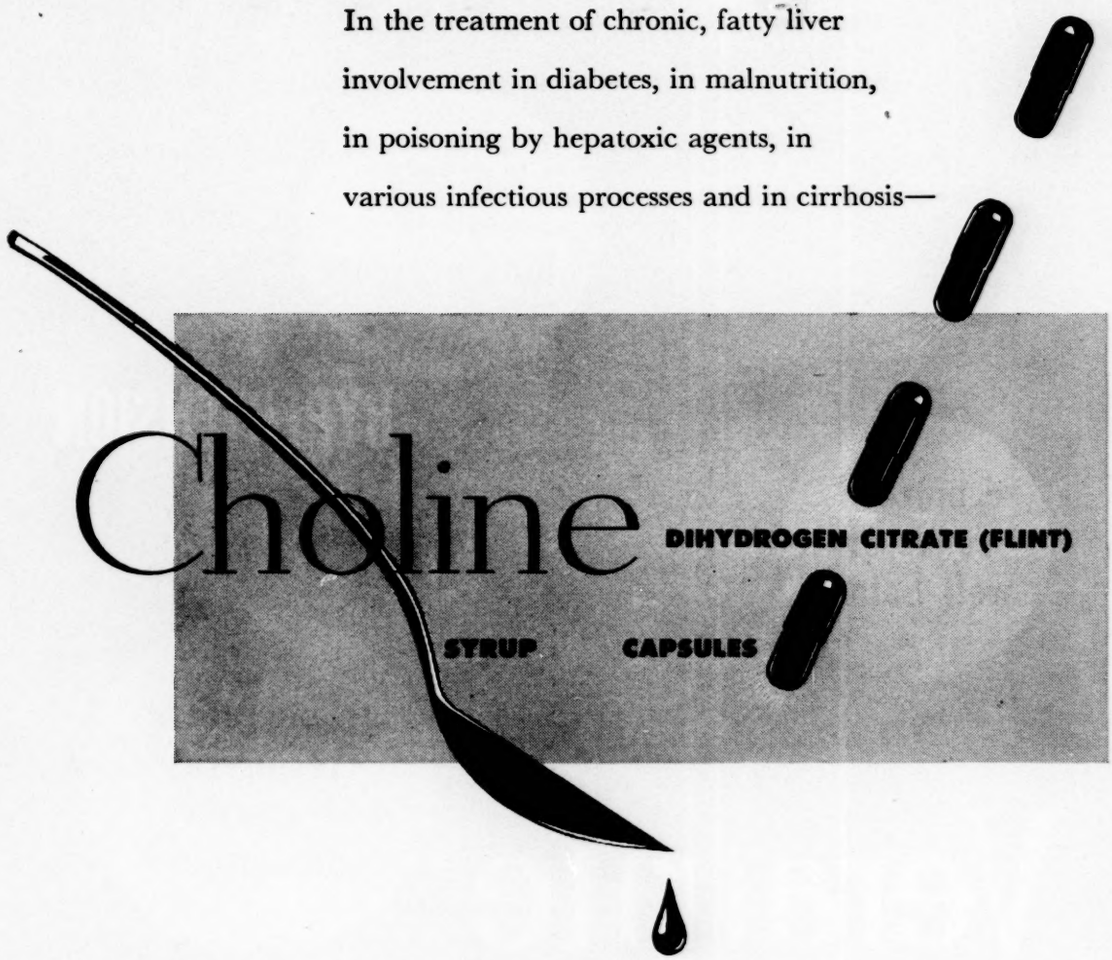

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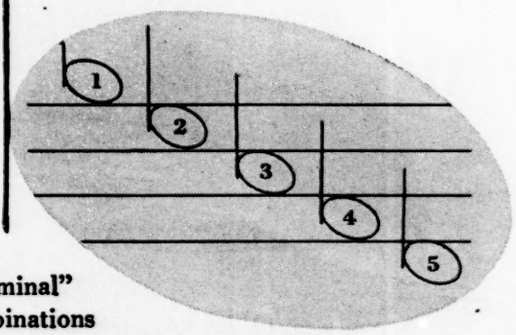

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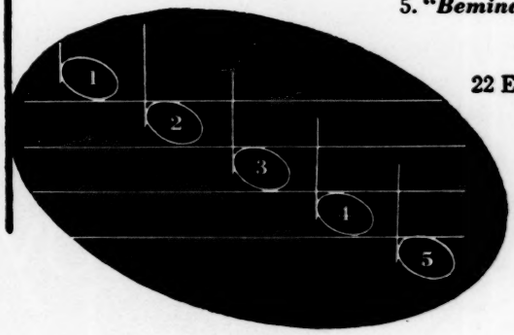

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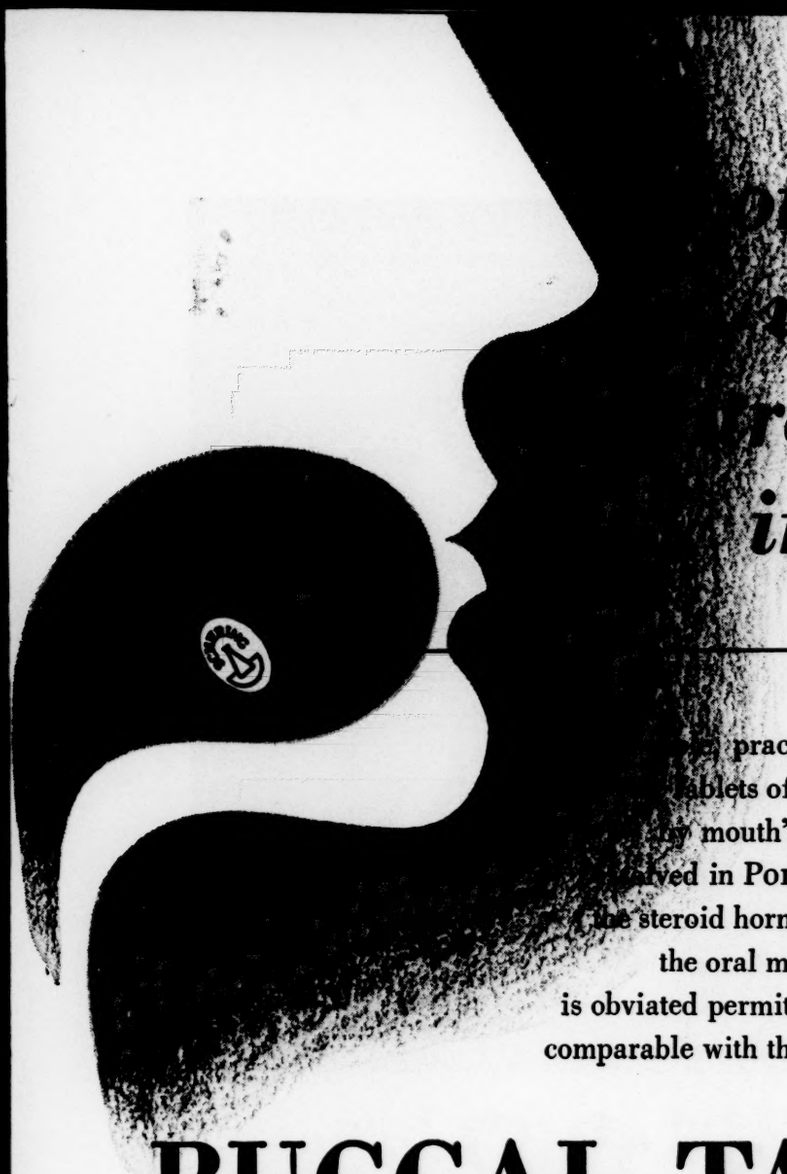

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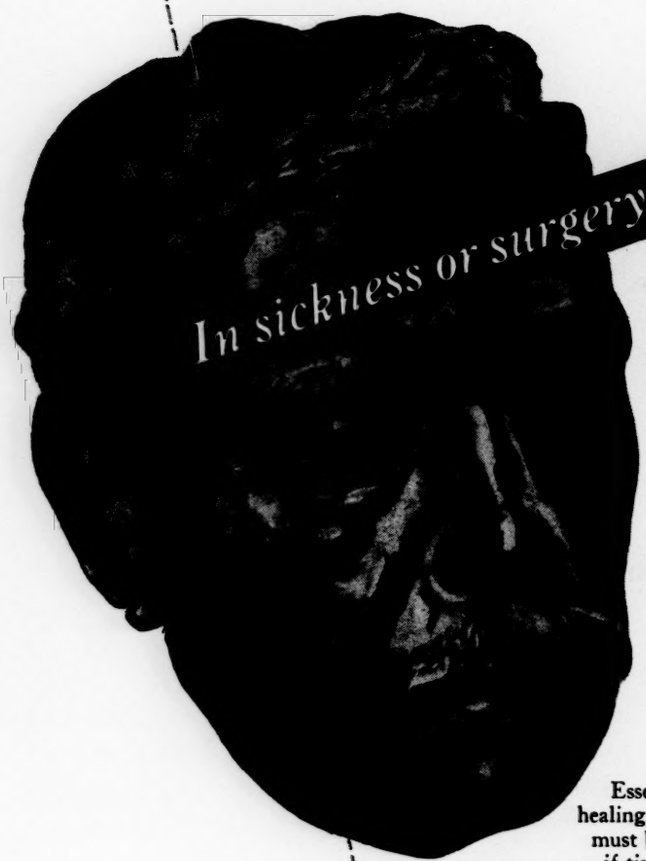

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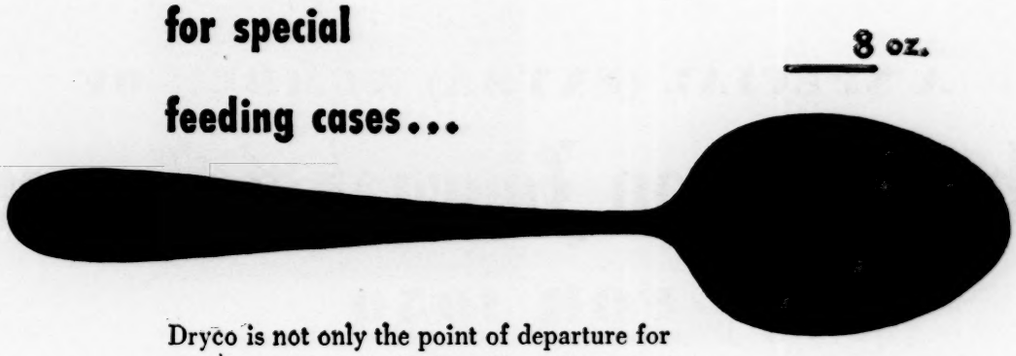

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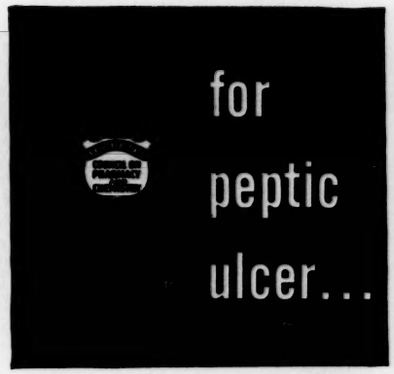

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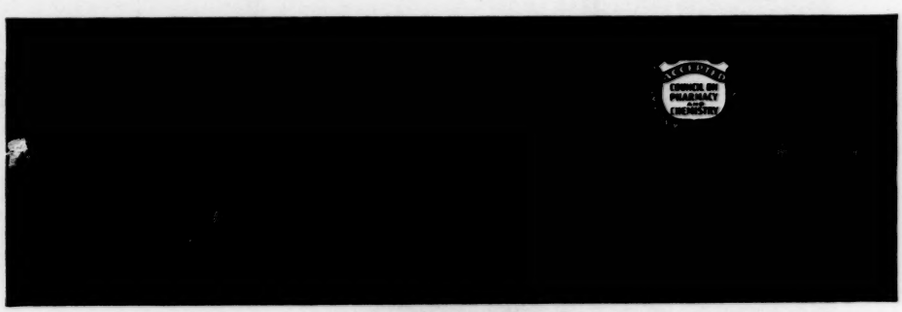

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